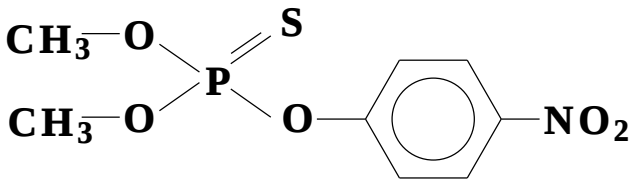
EVALUATION OF METHYL PARATHION AS A TOXIC AIR CONTAMINANT



Part C

Human Health Assessment

California Department of Pesticide Regulation
Medical Toxicology Branch

California Environmental Protection Agency
Sacramento, California

October 1999

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Department of Pesticide Regulation

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Methyl Parathion
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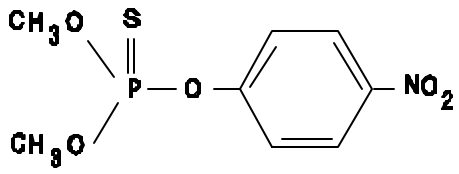

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Table of Contents

List of Tables

List of Figures

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1. SUMMARY

This Part C of the *Evaluation of Methyl Parathion as a Toxic Air Contaminant* contains the health risk assessment of the ambient air exposure to methyl parathion conducted under the Assembly Bill 1807, Toxic Air Contaminant Act. Methyl parathion is an organophosphate (OP) insecticide. It is oxidatively converted to methyl paraoxon through degradation in the environment and biotransformation in living organisms. Methyl paraoxon is responsible for the cholinergic neurotoxicities through the mechanism of cholinesterase (ChE) inhibition. Cholinesterase is an enzyme that hydrolyzes acetylcholine (ACh), a neurotransmitter at nerve synapses. In acutely OP toxic episodes, over abundance of ACh at effector sites, as a result of acetylcholinesterase (AChE) inhibition, is manifested through muscarinic and nicotinic, as well as the central nervous system (CNS) symptoms. Peripheral muscarinic effects can include increased intestinal motility, bronchial constriction, increased bronchial secretions, incontinence, miosis, secretory gland stimulation, hypertension, and bradycardia. Peripheral nicotinic effects include muscle weakness, "twitching", "cramps", and general fasciculations. Stimulation of muscarinic and nicotinic receptors in the central nervous system can cause headache, restlessness, insomnia, anxiety, slurred speech, tremors, ataxia, convulsions/seizures, depression of respiratory and circulatory centers, and coma. Death is usually due to respiratory failure from a combination of central and peripheral effects (i.e., respiratory center depression, ventilation muscles weakness and paralysis, excessive tracheobronchial secretions, bronchoconstriction). The most common treatments for the cholinergic crisis are atropine and ChE reactivators, such as oximes. Atropine and its analogs block the toxicological effects of excess ChE at muscarinic receptor sites. Oximes release and regenerate the ACh to its normal function before the ACh-methyl paraoxon complex becomes irreversible.

Methyl parathion is readily absorbed through oral, inhalation, and dermal routes of exposure. Pharmacokinetic data showed that the oral absorption is practically 100%. Acute toxicity data indicated that the absorption is comparable between the oral and inhalation routes. Therefore, no adjustment for route-specific absorption is necessary when oral toxicity data are used in characterizing the risk from inhalation exposures. Methyl parathion readily crosses the blood-brain barrier and the placenta. No significant amount of methyl parathion or its oxidated analogue was detected in milk, meat of goats and hens, and eggs of hens 8 hours after receiving oral administrations of methyl parathion. After oral exposure, the metabolites of methyl parathion are almost exclusively eliminated in the urine. Urinary *p*-nitrophenol has been used as a biomarker of exposure.

Among the commonly used laboratory animals, rats appeared to be the most sensitive species. The acute toxicity data showed that male rats could be more sensitive than the females. Neonates and young rats appeared to be more sensitive than the adults. Based on the data for median lethal dose and concentration, methyl parathion is determined to be a Category I oral toxicant, Category II inhalation toxicant, and Category IV eye and skin irritant. Methyl parathion is not shown to cause dermal sensitization.

A list of NOELs (No-Observed-Effect Levels) for the sensitive endpoints of methyl parathion were established for characterizing the risk of human exposures. A 30-day NOEL of 0.31 mg/kg/day was determined from a series of limited human studies focusing only on the effect of plasma and RBC ChE inhibitions. Other sensitive toxicity endpoints of methyl parathion identified in laboratory animals included brain ChE inhibition, cholinergic signs, neurobehavioral effects, and peripheral nerve demyelination. The NOEL for acute (a single) exposures was 0.025 mg/kg/day based on the inhibition of ChE in the plasma, red blood cells (RBC), and brain, and nerve demyelination occurred in rats. The subchronic NOEL was 0.029 mg/kg/day based on RBC ChE inhibition in rats. No NOELs for other sensitive endpoints can be directly determined from the animal studies. The LOEL (Lowest-Observed-Effect Level) was 0.03 mg/kg/day for plasma ChE inhibition in dogs, and 0.2 mg/kg/day for brain ChE inhibition and for neurobehavioral effects in rats. Using the default factor of 10, the respective estimated subchronic NOELs were 0.003 mg/kg/day and 0.02 mg/kg/day. The chronic NOEL was 0.02 mg/kg/day based on brain ChE inhibition and myelin degeneration in rats. A NOEL of 0.01 mg/kg/day based on the RBC ChE inhibition was estimated from the LOEL of 0.09 mg/kg/day in rats. Based on acute toxicity data in rats, a toxicity equivalence factor (TEF) of 10 for methyl paraoxon was used to account for the risk of methyl paraoxon concomitantly present with methyl parathion in the air.

Based on air monitoring studies in California, acute (a single day), seasonal (up to 9 months), and chronic (yearly) ambient air exposures were estimated for three representative population subgroups: children (6 years old), adult males, and adult females. The default breathing rates were 16.7 m^3/day for a 22.6 kg child, 21.4 m^3/day for a 76.9 kg adult male, and 11.4 m^3/day for a 62.4 kg adult female. The higher breathing rate per body weight of a child yielded higher exposures. Air monitoring data that resulted in the highest estimated exposures were used in this assessment to represent a realistic high end of exposures. The absorbed daily dosage (ADD) was the highest single day exposure. The seasonal average daily dosage (SADD) was the average exposure continuing for nine months. The annual average daily dosage (AADD) was a 9-month exposure amortized over a year. The respective potential ADD, SADD, and AADD were 64.55, 19.64, and 14.78 ng/kg/day for a 6 year old child. The respective ADDs at 17 and 20 feet from an application site were 1.26 and 0.56 Fg/kg/day for a 6 year old child. These values represented the total exposures of methyl parathion and methyl paraoxon as expressed in methyl parathion-equivalence.

Although methyl parathion is genotoxic in laboratory studies and there is some limited evidence of oncogenicity in rodent bioassays, the weight of evidence is insufficient for a quantitative assessment of oncogenic risk. The risk of methyl parathion exposures is characterized based on non-oncogenic effects and expressed in terms of a margin of exposure (MOE). MOE is the ratio of the NOEL to the exposure. Based on the estimated exposures, the MOEs for the acute ambient air exposure were 4,800 - 19,000 based on the human NOEL and 390 - 1,600 based on the NOEL for sensitive endpoints in rats but not examined in humans. The MOEs for the ambient seasonal exposures were 16,000 - 65,000 based on the human NOEL, 1,000 - 4,200 based on the NOEL for sensitive endpoints in rats, and 150 - 630 based on plasma ChE inhibition. The MOEs for ambient chronic

exposures were 1,300 - 5,400 based on the NOEL established in rats, and 670 - 2,700 based on RBC CHE inhibition in rats. For the exposures at the application site (17 and 20 yards from the rice field), the MOEs were 250 - 1,000 based on the human NOEL, and 20 - 80 (at 17 yards) based on the NOEL in rats.

The benchmark MOEs traditionally considered as adequate for the protection of human health are: a MOE of 10 based on a human NOEL and a MOE of 100 based on an animal NOEL. Thus, except for the seasonal MOE of 150 based on plasma ChE inhibition, the MOEs for the ambient air exposures are at least 7-fold greater than these benchmarks. The MOEs for 17 and 20 yards from the rice field are below the benchmark of 100 for the protection of human health.

It is essential that the MOEs are viewed in the context of the limitations and uncertainties presented in the risk appraisal section (Section 18.4). The key toxicity issues included: the severe limitations and adequacy of the 30-day human NOEL; the uncertainties associated with the critical NOEL for acute toxicity at 0.025 mg/kg/day; the default approach for estimating a NOEL from the LOEL; and the estimation of a TEF for methyl paraoxon based only on very limited acute toxicity data. The uncertainties in the exposure assessment included: the use of default physiological parameters and a point estimate approach; the limitation and adequacy of the monitoring data; and the assumption of a fixed ratio for methyl paraoxon to methyl parathion in the air (i.e., methyl paraoxon concentration was assumed to be 25% of the concentration of methyl parathion). The uncertainties in risk characterization included: the adequacy of information regarding the variation of sensitivity in human population including the current lack of data to account for the polymorphism of major detoxification enzyme paraoxonase (PON1) in the serum; the potential for higher sensitivity through pre- and post-natal exposures; and the future needs to realistically consider aggregate exposures (i.e., from all routes of exposure) and the cumulative risks (i.e., risk of multiple chemical exposures).

Reference concentrations of methyl parathion in the air were calculated based on the traditional benchmark MOEs for the protection of human health (a MOE of 10 based on a human NOEL and a MOE of 100 based on an animal NOEL). Accounting for the concomitant presence of methyl paraoxon, the 24-hour reference concentration of methyl parathion could be as low as 0.1 $\mu g/m^3$ (10 ppt) for the acute exposures and 0.01 to 0.08 $\mu g/m^3$ (1 to 8 ppt) for seasonal and chronic exposures. Additional considerations should be given to the demonstrated age-related sensitivity and the potential developmental neurotoxicity.

2. INTRODUCTION

Methyl parathion is an organophosphate (OP) insecticide. Its acute toxicity has been implicated in a number of occupational illnesses (USEPA, 1986a; DPR, 1997a). The toxicity and the widespread use of methyl parathion cause considerable concerns regarding its presence in the ambient air as a result of agricultural applications. The health risk assessment under AB1807, the Toxic Air Contaminant Act of 1983, is presented in this Part C of the Toxic Air Contaminant Evaluation (TACE) document.

Included in this document is a review of the toxicological database on methyl parathion, followed by an assessment of the risk to human health based on the reported ambient air concentrations as presented in Part B of the TAC document. The toxicological profile presented in this document includes information from both the studies on file at DPR and the pertinent publications in the open literature. The studies on file in DPR were submitted for fulfilling the pesticide registration data requirements under the Birth Defect Prevention Act of 1984 (Senate Bill 950; Petris). These studies were reviewed by the Medical Toxicology Branch both for identifying the potential adverse health effects and for the acceptability in filling data requirements based on Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) guidelines. Specific studies that were accepted for the SB950 data requirement purposes were so indicated in the text and in pertinent tables. The search for relevant publications in the open literature included the bibliographic electronic databases from Medline, Toxline and NTIS. The most recent database search was conducted in June 1999 and the June 1999 draft version of this document was updated accordingly. The revision also reflected further comments from the Scientific Review Panel for the TAC. A preliminary risk assessment document for the reregistration eligibility decision (RED) was published by USEPA in December, 1998. A revised RED was subsequently published in August, 1999 (USEPA, 1999). The critical toxicity thresholds established in the USEPA RED are highlighted in this document.

2.1. Recent Regulatory Activities

Methyl parathion has been used as a broad spectrum insecticide since its initial registration in 1954 (USEPA, 1986a). A Registration Standard was issued by USEPA in 1986. Together with other rice pesticides, methyl parathion was placed under reevaluation by DPR in 1991. The reevaluation was initiated due to concerns of its hazard to aquatic organisms, particularly, to the estuarine mysid *Neomysis mercedis* in the Sacramento-San Joaquin estuary. Completed in 1995, the reevaluation resulted in additional restrictions of its use to ensure that the rice field drainage discharge would not exceed the performance goal of 0.13 ppb.

Methyl parathion is not registered for indoor use. However, between 1991 and 1996, incidents of misuse occurred in Alabama, Arkansas, Illinois, Louisiana, Michigan, Mississippi, New York, Ohio, Tennessee, and Texas. Serious public health concerns were raised regarding the illegal application of “cotton poison” to exterminate roaches and other pests in residential homes, day care facilities,

restaurants, and hotels in these states. The Agency for Toxic Substances and Disease Registry (ATSDR) and USEPA jointly issued a nationwide alert in December 1996 to inform and address some of these issues. To date, actions taken to reduce and mitigate risks included: clean up operations under USEPA Superfund programs, recall the EC (emulsifiable concentrate) formulation by the manufacturer, addition of an odor agent, tamper-resistant packaging, and stepped-up communication.

In September 1997, methyl parathion was added to the worldwide list of hazardous pesticides under the Prior Informed Consent (PIC) procedure. The voluntary PIC is jointly administered by the United Nations (UN) Food and Agriculture Organization (FAO) and UN Environment Programme (UNEP). Chemicals in the list should not be exported without the agreement of the importing country. The action was based on the high toxicity and the potential health risk particularly under the conditions of use in developing countries. Negotiations are in progress to change the voluntary PIC procedure to a legally binding convention.

Current to the June 1999 draft document, 30 pesticide products containing methyl parathion are registered for use nationwide. Included in the 5 products currently registered in California are formulations containing 20-54.5% methyl parathion, and a product in microencapsulation form (22%). In California, methyl parathion can be applied through aerial application, ground spray or broadcast application, applied to soil (injection or work into soil), or as a water application. A human health assessment of methyl parathion was published by USEPA in August 2, 1999 (USEPA, 1999) for making reregistration eligibility decisions and for tolerance reassessments consistent with the Federal Food, Drug, and Cosmetic Act (FFDCA) as amended by the Food Quality Protection Act of 1996 (FQPA). On the same day, USEPA also announced the voluntary cancellation of many uses as an effort to reduce human risks from methyl parathion exposures. The canceled food uses included use on all fruits (apples, peaches, pears, grapes, nectarines, cherries, plums), and many vegetable crops (carrots, succulent peas, succulent beans, tomatoes, artichokes, broccoli, brussels sprouts, cauliflower, celery, collards, kale, kohlrabi, lettuce, mustard greens, rutabagas, spinach, turnips). The canceled non-food uses included ornamentals, grasses grown for seed, mosquito use, and nursery stock. The existing stocks with the canceled crop use may be applied until December 31, 1999. It should be noted that, in this DPR risk assessment document, air monitoring data was taken from rice field applications, the use of which is retained in the current label.

2.2. Mechanisms of Toxicity

The mechanism of cholinesterase (ChE) enzyme inhibition has been well documented for the cholinergic neurotoxicities of organophosphates, including methyl parathion. However, a direct involvement of ChE inhibition is not apparent for many other neurological manifestations of OPs, particularly the neuropsychological and/or neurobehavioral effects of the central nervous system. Recent investigations on the biochemical modulations of OPs in the brain represent some initial efforts in defining alternative

mechanisms of OP actions. These preliminary findings are also presented in this document to provide further understanding of toxicities not apparently correlated to the levels of ChE inhibition.

2.2.1. Cholinesterase inhibition

ChEs are a family of enzymes found throughout the body that hydrolyze choline esters. In the nervous system, acetylcholinesterase (AChE) is involved in the termination of impulses across nerve synapses including neuromuscular junctions by rapidly hydrolyzing the neural transmitter, acetylcholine. The oxygen analog of methyl parathion inhibits ChE through binding to the active site of ChE and forming a phosphoryl-cholinesterase complex. Initially, the complex is reversible, but over time, the complex may undergo "aging" (forming dealkylated phosphorylated ChE) and become refractory to ChE reactivation (Chambers and Chambers, 1989).

Inhibition of AChE leads to accumulation of acetylcholine (ACh) in the synaptic cleft, resulting in over-stimulation of the nerves followed by depression or paralysis of the cholinergic nerves throughout the central and peripheral nervous system. AChE is highly selective, although not exclusively, for acetyl esters as substrates (Brimijoin, 1992). Butyrylcholinesterase (BuChE) is another form of cholinesterase which preferentially hydrolyzes butyryl and propionyl esters, depending on the species. However, it will also hydrolyze a wider range of esters, including acetylcholine (Brimijoin, 1992). Unlike AChE, the physiological function of BuChE in the nervous system is not known.

AChE and BuChE are found in most tissues. AChE is the predominant form of ChE in the central nervous system and in the neuromuscular junctions of peripheral tissues, such as the diaphragm, skeletal muscle, heart, and spleen (Gupta *et al*., 1991; Mendoza, 1976). AChE and BuChE are present in roughly equal proportions in the liver and kidney. Non-synaptic AChE is also present to a lesser extent in peripheral tissues; however, its function is not known (Brimijoin, 1992). Non-synaptic AChE is essentially the only ChE present in erythrocytes of higher animals. Although BuChE is the predominant form of ChE in the plasma of humans, the ratio of AChE to BuChE in the plasma varies greatly from species to species and between sexes. For example, the AChE to BuChE ratio in human plasma is approximately 1 to1000, but closer to 1 to 2 in female rats and 3 to 1 in male rats.

In acutely OP toxic episodes, over abundance of ACh at effector sites, as a result of AChE inhibition, is manifested through muscarinic and nicotinic, as well as the central nervous system (CNS) symptoms (Murphy, 1986; Ecobichon, 1994). Peripheral muscarinic effects can include increased intestinal motility, bronchial constriction, increased bronchial secretions, incontinence, miosis, secretory gland stimulation, hypertension, and bradycardia. Peripheral nicotinic effects include muscle weakness, “twitching”, “cramps”, and general fasciculations. Stimulation of muscarinic and nicotinic receptors in the central nervous system can cause headache, restlessness, insomnia, anxiety, slurred speech, tremors, ataxia, convulsions/seizures, depression of respiratory and circulatory centers, and coma. Death is usually due to respiratory failure from a combination of central and peripheral effects (i.e.,

respiratory center depression, ventilation muscles weakness and paralysis, excessive tracheobronchial secretions, bronchoconstriction).

Depending on the extent of inhibition, the plasma ChE level may return to normal within several days. The RBC ChE level can, however, remain depressed for an extended period because RBC ChE remains inhibited for the life of an erythrocyte which is approximately four months in humans (Murphy, 1986).

2.2.2. Aliesterase phosphorylation

Aliesterase (carboxylesterase, B-esterase) is predominant in the liver and plasma. The interactions of OPs with aliesterase can affect their inhibition of ChE and potentially result in a reduction of cholinergic toxicity of OPs. The extent of protection varies according to the pattern and predominance of biochemical interactions at a particular tissue site and for a specific OP. Chambers and Carr (1993) compared the interaction of three OPs (ethyl parathion, methyl parathion, chlorpyrifos) and their oxons with brain AChE (cerebral cortex and medulla oblongata) and with liver and plasma aliesterase. The time course of AChE and aliesterase inhibition in male and female Sprague-Dawley-derived rats was characterized by measurements of enzyme activities at 2 hours, 1, 2, and 4 days after a single intraperitoneal (i.p.) injection of the OPs. With respect to the time to peak activities and the level of inhibition, the aliesterase inhibition pathway by methyl parathion and methyl paraoxon was either comparable or slightly less prominent than the AChE inhibition. Conversely, the aliesterase inhibition by ethyl parathion and chlorpyrifos and their oxons had shorter lag times and/or higher peaks than the AChE inhibition. In this comparison, the author speculated that, the less prominent interaction by methyl parathion and its oxon with aliesterase may imply lesser protection by this pathway. However, it is important to note that toxicity is often the integration of a number of biochemical interactions and pathways that involve activation and/or detoxification rather than solely dependent on the kinetics of a single pathway. Therefore, a lesser degree of protection by aliesterase to methyl parathion does not necessarily mean a higher overall toxicity. In fact, ethyl parathion is generally more acutely toxic than methyl parathion. The importance of the detoxification pathway to the overall toxicity of an OP was illustrated through a comparison of ChE and aliesterase interactions and the glutathione conjugation activities in mosquito fish for the three OPs (Boone and Chambers, 1996). As in the rats, the potential of aliesterase protection was also found to be less for the toxicity of methyl parathion than for ethyl parathion or chlorpyrifos. On the other hand, the reduction in hepatic nonprotein sulfhydryl contents was greater and more prolonged after the exposure to methyl parathion than to the other two OPs. These results suggested that glutathione conjugation instead of aliesterase interaction may have greater contribution to the overall lower acute toxicity of methyl parathion in mosquito fish (e.g., the LD_{50} for methyl parathion was 40-fold higher than for ethyl parathion).

The dynamic interactions of various metabolic pathways and their corresponding kinetics can also be the underlying reasons for the inter-species and inter-individual variation of sensitivities to OPs. These topics specific to methyl parathion are presented in Section 5., *Toxicity Variation and Modification*.

2.2.3. Mechanisms other than ChE inhibition

Recent investigations revealed several possible alternative mechanisms to the ChE inhibition. These studies were aimed at defining the general mechanisms for OPs rather than for a specific OP. Only a brief summary of the general information is presented in this section, while greater details are given for studies in which methyl parathion was specifically used in the investigation.

Several OPs were shown to interact directly with cholinergic receptors in a manner that is either noncompetitively modulating the muscarinic receptor binding or competitively interacting with radioligands for cholinergic receptors (Chaudhuri *et al*., 1993). In a recent study, Ward and Munday (1996) showed that the active forms of some OPs (e.g., paraoxon, malaoxon, chlorpyrifos oxon) bound directly to the cholinergic muscarinic receptors in the rat brain. Paraoxon was also shown to modulate selective ligand-gated ion channels in cultured rat hippocampal neurons (Rocha *et al*., 1996).

In an *in vitro* study using fluorescence probes, Pala *et al*. (1991) reported that methyl parathion, ethyl parathion, and malathion inhibited the activity of calmodulin and altered its conformation. Calmodulin is a low molecular weight Ca^{++}-binding protein abundantly present in the brain. It is an intracellular calcium ion receptor which regulates many Ca^{++}-dependent processes. The authors speculated that conformational changes to calmodulin may additionally contribute to the neurotoxicity of OPs. Nayeemunnisa and Begum (1992) studied the effects of methyl parathion on the regulatory proteins in the developing brain of 15 and 21 days old rat pups that received a single intraperitoneal injection of 0.66 or 1 mg/kg methyl parathion. Methyl parathion treatment resulted in changes in calmodulin levels (a decrease in the cerebellum and an increase in the brain stem), a decrease in Ca^{++}-ATPase, and an increase in phospholipids in four regions of the CNS (cerebral cortex, brain stem, cerebellum, spinal cord). Liu *et al*. (1994) studied the effects of methyl parathion and malathion on Ca^{++}-channels in bovine chromaffin cell cultures. Both OPs showed a dose-dependent inhibition of both catecholamine secretion and Ca^{++} uptake induced by either 1,1-dimethyl-4-phenylpiperazinium (DMPP) or high K^{+} environment. The authors speculated that these effects could be mediated through inhibiting the voltage-gated Ca^{++}-channels on the plasma membrane and that the chain of events might be a plausible alternative to the mechanism through ChE inhibition.

2.3. Treatments for Poisoning

Atropine and AChE reactivators (e.g., oximes) are the most common treatments for the cholinergic crisis resulting from the over-stimulation by the excess ACh at effector sites. Atropine and its analogs

block the toxicological effects of excess ACh at muscarinic receptor sites but are ineffective on neuromuscular junctions of skeletal muscles (Gallo and Lawryk, 1991). Scopolamine has also been used for managing OP poisoning but is generally less effective than atropine. General description of the pharmaceutical properties of oximes was published by Laws (1991) and Howland and Aaron (1998). The predominant mechanism of oximes is in reactivating the inhibited cholinesterase before the phosphorylated enzyme undergoes aging. Pralidoxime (2-pyridine aldoxime methyl, or 2-PAM) releases and regenerates the AChE to its normal function. Although the molecular consideration for 2-PAM as a quaternary nitrogen derivative would not predict a ready access of 2-PAM across the blood brain barrier (BBB), clinical observations showed its action in the CNS (Howland and Aaron, 1998; Schexnayder *et al.*, 1998). Alternatively, pro-2-PAM, the dihydropyridine derivative of 2-PAM, has been used to improve the passage through membrances such as BBB (Howland and Aaron, 1998). Diacetyl monoxime (DAM) is also effective on the central nervous system. In addition to ChE reactivation, oximes (e.g., 2-PAM, DAM) were also shown to inactivate OPs by reacting directly with the OP molecule. Other interactions of oximes that have less defined therapeutic applications in acute OP poisoning included anticholinergic effects and depolarization of the neuromuscular junction. Animal studies and therapeutic treatment of severe poisoning cases showed evidence of synergistic effects between atropine and oximes.

The rate of phosphorylated ChE reactivation by oximes varies between OPs and with the different physiological and toxicological conditions. Information specific to methyl parathion is presented below. Willems *et al.* (1993) evaluated the therapeutic effects of oximes in seven patients in Belgium poisoned by ethyl and methyl parathion. The authors reported that the effectiveness of oximes in reactivating ChE was dependant not only on the plasma concentration of oximes but also on the serum concentrations of the OPs. Without an apparent delineation in the presented data, the authors concluded that oximes appeared to be ineffective in reactivating ChE when the plasma OP concentration was above 30 Fg/l. Uehara *et al.* (1993) conducted an *in vitro* study to compare the 2-PAM reactivation of plasma and RBC ChE that was inhibited by three OPs. Plasma and RBC samples were obtained from male Sprague-Dawley rats 3 to 48 hours after receiving an oral intubation of 400 mg/kg fenitrothion, 3 mg/kg methyl parathion, or 5 mg/kg ethyl parathion. As expected with the aging of the phosphoryl-ChE complex, the RBC and brain ChE reactivation rates decreased in time. By 48 hours, the reactivation of RBC and brain ChE inhibited by fenitrothion and methyl parathion was reduced to approximately 10-20% while the rate was still high (approximately 65-80%) for ChE inhibited by ethyl parathion. The difference in the reactivation rate was statistically different starting at 6 - 12 hours after the OP treatment. The authors concluded that the aging appeared to be specific to the alkyl moiety of the OPs, with dimethyl moiety causing faster aging than a diethyl moiety.

3. PHARMACOKINETICS

Information on the pharmacokinetics in both test animals and humans enhances the understanding of toxicological effects of methyl parathion and is essential for a relevant extrapolation of data from

laboratory animals to humans and from one route of exposure to another. Information on the absorption, distribution, metabolism, elimination of methyl parathion from various routes of exposure is presented.

3.1. Absorption

Methyl parathion is readily absorbed through oral, inhalation, and dermal routes of exposure. Pharmacokinetic studies are not available for a direct determination of the absorption from dermal and inhalation routes. Data supporting an estimation of the absorption factor from these two routes are presented.

3.1.1. Oral route

Absorption from the gastrointestinal tract is both rapid and nearly complete. Based on the available data in rats, mice, and dogs presented in this section, a practical level of 100% oral absorption was determined.

Methyl parathion was detected in the plasma and brain of rats 6–8 minutes after a lethal oral dose of 50 mg/kg (Yamamoto *et al.*, 1983). In rats that received a lower single oral dose of 1.5 mg/kg methyl parathion and in guinea pigs that received a single oral dose of 50 mg/kg methyl parathion, the maximum plasma and brain ChE inhibition was reached in 30 minutes (Miyamoto *et al.*, 1963b). Braeckman *et al.* (1983) reported that the time to the peak blood concentration of methyl parathion in dogs varied between 2 to 9 hours after an oral administration at 20 mg/kg. On the other hand, a much shorter time-to-peak concentration of methyl paraoxon was reported by De Schryver *et al*. (1987). The peak level was reached within 3–16 minutes in dogs that received an oral administration of 15 mg/kg methyl paraoxon.

Since orally administered methyl parathion is primarily excreted in the urine, the total oral absorption can be estimated based on the recovery of radioactivity in the urine. Using ^{32}P-methyl parathion, Miyamoto *et al.* (1963a) reported an approximately 70% recovery of radioactivity in the urine within 48 hours after a single gavage dosing of 1.5 mg/kg ^{32}P-methyl parathion to rats or 50 mg/kg ^{32}P-methyl parathion to guinea pigs. A slightly higher recovery was reported in mice by Hollingworth *et al.* (1967). Approximately 85% of orally administered 3–7 mg/kg ^{32}P-methyl parathion was recovered in the urine.

A pharmacokinetic study in Wistar rats by Van Dijk (1988a) was available on file in DPR. Data from this study also showed a high urinary recovery (>83%) after oral administration. In this study, male and female rats were either given a single oral intubation of 0.5 or 2.5 mg/kg ^{14}C-methyl parathion or 0.5 mg/kg ^{14}C-methyl parathion (uniformly labeled on the phenol ring) after 14 days of pre-loading with non-radiolabeled methyl parathion. The total recovery of radioactivity was 95.6-104.2%. Adjusted

for a 100% mass balance, the average (N=5) percentage of administered radioactivity recovered in the urine within 48 hours were 83.6% (females) and 95.2% (males) at 0.5 mg/kg methyl parathion and 79% (females) and 89.9% (males) at 2.5 mg/kg methyl parathion. The urinary recovery after 14-day pre-loading was in the same range for the males (93%) but higher for the females (91.6%). At least 82% of the total urinary excretion was completed by 8 hours of dosing. Approximately 87-95% of the urinary excretion was in the form of sulphate or glucuronide conjugate of *p*-nitrophenol. Adjusted for 100% recovery, the 48-hour radioactivity recovered in the feces was 3.3-9.6% of the administered activity. At the end of the 48 hours, very little radioactivity was left in the intestinal tract (up to 0.3%), carcass (up to 0.3%), and organ/tissues (up to 0.1%). Less than 0.01% was recovered in the expired air in males that received 2.5 mg/kg methyl parathion.

The oral absorption can also be estimated by comparing the urinary recovery from intravenous (i.v.) and from oral dosing. The estimated oral absorption was 77-79% in dogs that received 3 mg/kg ^{35}S-methyl parathion (Braeckman *et al.*, 1983) and 67% in dogs that received 15 mg/kg ^{35}S-methyl paraoxon (De Schryver *et al.*, 1987). The same range of values was also reflected in the ratio of the oral and i.v. LD_{50} values derived from within a same report (Table 1 in Section 6.2.1). The ratio of the i.v. LD_{50} to oral LD_{50} was 75-81% in rats from the report by Newell and Dilley (1978) and 76% in mice from the report by Miyamoto *et al.* (1963b).

3.1.2. Dermal route

Dermal contact is the predominant route of occupational exposures from the pesticidal use of methyl parathion. Dermal absorption is evident in the detection of methyl parathion in the blood, *p*-nitrophenol in the urine, and ChE inhibition among agricultural workers (Nemec *et al.*, 1968; Ware *et al.*, 1974). Using a human skin diffusion cell apparatus, Sartorelli *et al.* (1997) compared the movement of methyl parathion across the skin between the acetone vehicle and the water-diluted commercial formulation that resulted in a water-to-xylene ratio of 99-to-1. The results showed a greater skin absorption from the water preparation. The respective penetrations after 24 and 48 hours were 1.35 and 3.58% in acetone and 5.20 and 8.99% in water.

In the absence of *in vivo* data specific to methyl parathion, dermal absorption pattern of ethyl parathion may be considered as a surrogate for methyl parathion. Gyrd-Hansen *et al.* (1993) studied the effects of vehicles in the percutaneous absorption of OPs in pigs. Based on the serum concentrations, the estimated percutaneous absorption of ethyl parathion within 10 days of a single dermal exposure at 1 mg/kg was 15-30% with dimethyl sulphoxide (DMSO) or 1-octanol as the vehicle. A much lower absorption of 4-5% was reported when Carbowax® (marcogol 400) vehicle was used (Gyrd-Hansen and Rasmussen, 1993). Using absolute ethyl alcohol as a vehicle, Qiao and Riviere (1995) studied the effects of occlusion and sites on the dermal absorption of parathion in swine (300 Fg ethyl parathion applied to 7.5 cm^2 skin surface). Occlusion substantially enhanced the absorption. The respective absorptions for the abdominal and back skins were approximately 8% and 25% when non-occluded

and 44% and 49% when occluded. Using a model of bioavailability-excretion analysis, the authors estimated that the cutaneous metabolism would substantially contribute to the marked increase in absorption by occlusion (Qiao and Riviere, 1995). In humans, the absorption of topically applied ethyl parathion was estimated to be approximately 10–30% in 24 hours (Oudiz and Klein, 1988). The same range of values was also indicated in the comparison of the oral and dermal LD_{50} derived from within the same report. The ratio of the oral LD_{50} to the dermal LD_{50} in rats from the reports by Gaines (1969) and by Newell and Dilley (1978) was 11-36% (the LD_{50} values are listed in Table 1; under Section 6.2.1).

3.1.3. Inhalation route

Without proper respiratory protection, inhalation of methyl parathion can also be a significant route of occupational exposure. Hartwell and Hayes (1965) and Newell and Dilley (1978) reported that formulating plant workers protected by respirators had fewer cases of ChE inhibition and poisonings from exposures to methyl parathion.

Data from direct measurements of inhalation absorption are not available. The absorption can be estimated by a comparison of the i.v. LD_{50} and the inhalation LC_{50} derived from within the same report. Newell and Dilley (1978) reported an i.v. LD_{50} of 9–14.5 mg/kg/day and a 1-hour inhalation LC_{50} of 257-287 mg/m^3 in rats (see: Table 1 in Section 6.2.1). Using the DPR current default respiratory rate of 0.96 m³/kg/day (or 0.04 m^3/kg/hr) for rats, the estimated exposure at the inhalation LC_{50} was 10–12 mg/kg (i.e., the LC_{50} multiplied by 0.04 m^3/kg/hr). This level was within the same range as the i.v. LD_{50}. The inhalation absorption can also be estimated based on the comparison of the exposure levels that would achieve the same level of ChE inhibition. Newell and Dilley (1978) reported a 41% whole blood ChE inhibition (i.e., the inhibition dose at 41%, or the ID_{41}) one hour after either an oral exposure at 11.7 mg/kg or an inhalation exposure at 264 mg/m^3 (see: Table 3 in Section 6.2.2). Using the DPR current default respiratory rate of 0.96 m³/kg/day (or 0.04 m^3/kg/hr) for rats, the estimated inhalation exposure was 10.6 mg/kg (i.e., 264 mg/m^3 multiplied by 0.04 m^3/kg/hr). This exposure level was also within the same range of the oral exposure level at 11.7 mg/kg. The above two comparisons (i.e., the i.v. LD_{50} versus LC_{50} and the IC_{41} from i.v. versus inhalation routes) support the conclusion that the absorption of methyl parathion through inhalation can practically be considered as comparable to the absorption through the oral route. Therefore, no adjustment of route-specific absorption was necessary in characterizing the risk from inhalation exposure using threshold levels determined from oral studies.

3.2. Distribution

Information on the distribution of methyl parathion and its metabolites to tissues and organs of laboratory animals is available in the open literature. Studies in rats and guinea pigs showed that methyl parathion readily crosses the blood-brain barrier. Transplacental transport of methyl parathion has also been in rats and in an *in vitro* human placental perfusion study. No significant amount of methyl parathion or its oxidated analogue was detected in milk, meat of goats and hens and the eggs of hens 8 hours after receiving oral administrations of methyl parathion.

3.2.1. Tissues and organs

Braeckman *et al.* (1980) studied the pharmacokinetics of methyl parathion in dogs that received 1, 3, 10 or 30 mg/kg methyl parathion intravenously. A correlation analysis of serum concentration per unit dose over 30 hours revealed no dose dependency within the range of methyl parathion tested, including the lethal level of 30 mg/kg. The time-course of serum concentrations of six dogs that received 10 mg/kg methyl parathion can be described by a quadratic or cubic polynomial equation. Except for one dog that did not yield harmonic estimates, the mean terminal half life ($t_{½}$) was 7.2 hours (ranging from 6.6 to 8.8 hours), and the mean volume of distribution was 9.6 *l*/kg (ranging from 4.6 to 12.8 *l*/kg). The authors speculated that the large volume of distribution could be due to high tissue distribution in a peripheral compartment despite the extensive plasma protein binding. Based on the comparison of the area-under-the-curve in plasma concentration-time plots following i.v. injection, Braeckman *et al.* (1983) reported a relatively lower systemic availability in dogs following oral exposure. The concentration of methyl parathion in the hepatic vein was much lower than in the femoral artery. The authors speculated that high hepatic extraction might have contributed substantially to the low systemic availability after oral exposures (Braeckman *et al.*, 1983).

Methyl parathion has been detected in various tissues of animals after exposure. Within 2.5 minutes after an i.v. administration of ^{32}P-methyl parathion (>98% pure) to rats (at 15 mg/kg) and guinea pigs (at 20 and 40 mg/kg), the radiolabel was detected in many tissues, among which the liver, lung, kidney, brain, and heart had the highest radioactivity (Miyamoto, 1964).

3.2.2. Blood-brain barrier

Methyl parathion readily crosses the blood-brain barrier. It was detected in the brain of Wistar rats within 6 to 8 minutes after oral dosing at 50 mg/kg and 90 seconds after i.v. injection at 3 mg/kg (Yamamoto *et al.*, 1983). In rats that received 1.5 mg/kg ^{32}P-methyl parathion via gavage, peak concentrations in both brain and blood (whole) were reached at 3 hours after dosing (Miyamoto *et al.*, 1963a). Similarly, in guinea pigs that received 50 mg/kg ^{32}P-methyl parathion via gavage, the peak concentrations in both brain and blood (whole) were achieved at 1 hour of dosing (Miyamoto *et al.*, 1963b).

3.2.3. Transplacental transport

Transplacental transport of methyl parathion has been studied in rats. A reduction of brain ChE was detected histochemically in fetal brain of pregnant Holzmann rats given a single i.p. injection of 4-6 mg/kg methyl parathion (ethanol-propylene glycol vehicle) on day 9 or 15 of gestation (Fish, 1966). Methyl parathion was detected in both the placenta and fetal liver, brain, and muscle tissues following oral administrations to pregnant rats (Ackermann and Engst, 1970). In an *in vitro* human placental perfusion study with parathion and methyl parathion, Benjaminov *et al.* (1992) reported the inhibition of

placental AChE and the presence of "parathion" in both the placental tissue and the fetal reservoir. Unfortunately, the report did not differentiate between "parathion" and "methyl parathion" in the residue analysis by gas chromatography. It may be assumed that residues of "methyl parathion" instead of "parathion" were detected since the report specified that the former was used as an internal standard for residue extraction.

3.2.4. Milk, meat, and eggs

USEPA does not establish tolerances for methyl parathion in milk, meat, and eggs (CFR, 1997). Neither does the Codex Committee for Pesticide Residues establish maximum residue limits (MRLs) for these commodities. Tolerances and MRLs are the highest level of residues permitted to be present in agricultural commodities. Concerns of the widespread use of OPs in the livestock industry in Portugal prompted Lino and Silveira (1992) to monitor the residues of *cis*-mevinphos, methyl parathion, and paraoxon in 25 milk samples taken from commercial circles. No residues of mevinphos or methyl parathion were detected (detection limits of 0.5 ppb for *cis*-mevinphos, 1.0 ppb for methyl parathion). Of the 25 samples, 22 had residues of paraoxon ranging from 1.5 to 8.7 ppb (ave. 3.6 ppb).

Three animal residue studies on methyl parathion are available: two residue studies by Van Dijk were conducted in hens (Van Dijk, 1988b) and in goats (Van Dijk, 1988c), and one study by Baynes and Bowen (1995) was conducted in goats. In the two studies by Van Dijk (1988b, 1988c) using ^{14}C-methyl parathion, radioactivity in tissues and fluids was expressed as the equivalent amount of the parent compound. Samples of chicken eggs and goat milk were collected at two intervals daily; 8 hours after dosing and prior to the next dosing. Blood samples were taken at various intervals. In the hen study (Van Dijk, 1988b), laying hens were intubated with 0.5 mg/kg ^{14}C-methyl parathion for 1 or 3 days. The highest plasma radioactivity was noted 4 hours after a single dosing. The total average radioactivity in edible portions and in the blood of 5 hens was approximately 2% of the total administered radioactivity. The highest radioactivity of 0.03 Fg/g in eggs was found after the 3rd dosing. The level was less than 0.1% of the administered radioactivity. In the goat study, one 60 kg lactating goat was intubated with a mean daily dose of 0.58 mg/kg/day ^{14}C-methyl parathion for 3 days and sacrificed one hour after the last dosing (Van Dijk, 1988c). The highest plasma concentration of 0.174 Fg/g was noted 1 hour after the first dosing. The highest radioactivity of 0.036 Fg/g in the milk was found 8 hours after the second dosing. The respective radioactivities in the milk prior to the second and third dosing were 0.008 and 0.012 Fg/g. Desmethyl paraoxon and amino methyl paraoxon each constituted approximately 34-38% of the total radioactivity in the milk 8 hours after the second dosing. Assuming a daily milk production of approximately 2 liters, the radioactivity in milk was less than 0.2% of the total administered radioactivity.

In the study by Baynes and Bowen (1995), four lactating goats received methyl parathion either by gelatin capsules (5 mg/kg/day, 3 days) or through i.v. injection (5 mg/kg, a single dose). Neither methyl parathion nor its metabolites (i.e., methyl paraoxon, methyl phosphate, or methyl thiophosphate)

was detected in the milk, plasma or urine. The respective minimum detection limits (MDLs) for these 4 compounds were 0.011, 0.015, 0.074, and 0.204 Fg/ml. The average volume of distribution of 5.24 *l*/kg was determined from the 4 goats that received the i.v. injection.

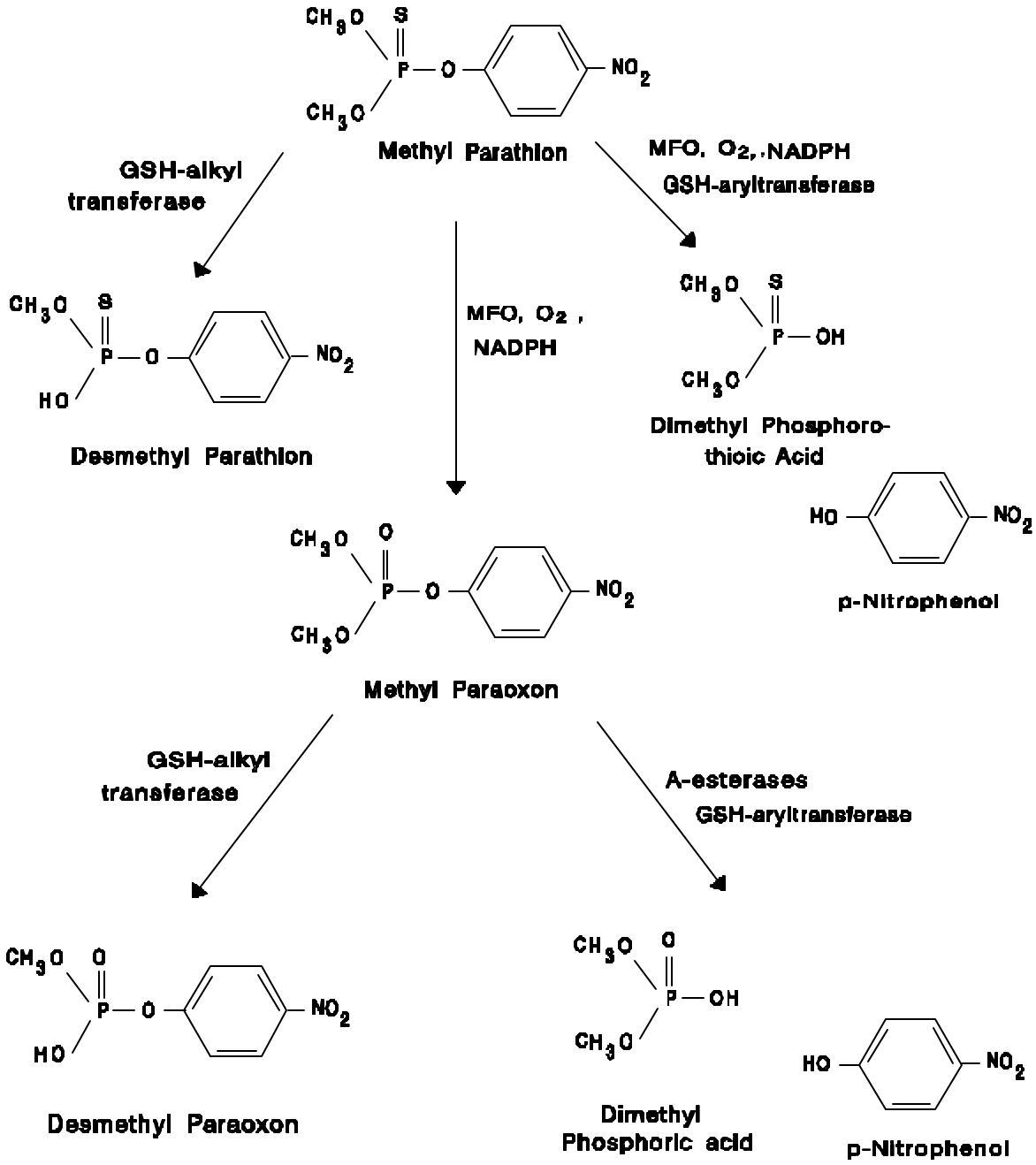
3.3. Metabolism

The major pathways of metabolic transformation for methyl parathion are shown in Figure 1. The parent compound is activated to its oxidated analog (i.e., from methyl parathion to methyl paraoxon) through oxidative desulfuration by the mixed function oxidase (MFO), or cytochrome P450, system (Hollingworth *et al.*, 1967; Menzie, 1969; Murphy, 1982; Rao and McKinley, 1969). The oxidative products, not the demethylation product, were shown to inhibit ChE (Rao and McKinley (1969). Oxidative activation has been demonstrated using cellular preparations from the liver and brain and *in situ* in the lung (Forsyth and Chambers, 1989; Lessire *et al*., 1996). Alternatively, a recent study using partially purified rat brain preparation, de Lima *et al.* (1996) demonstrated the activation of methyl parathion through a pathway that did not involve the Cytochrome P450 system. The activated product, capable of inhibiting AChE, appeared to be structurally very similar to methyl parathion. The authors speculated that it could be a methyl parathion isomer.

Detoxification of methyl parathion and methyl paraoxon is accomplished through dearylation or demethylation by glutathione (GSH)-dependent aryl- or alkyl-transferases. It is interesting to note that although demethylation might be an important route of detoxification for methyl parathion and methyl paraoxon, the closely related ethyl parathion and ethyl paraoxon showed very little dealkylation (Benke and Murphy, 1975; Benke *et al.*, 1974; Hollingworth *et al., 1967).* GSH-dependent demethylation has also been demonstrated in an *in vitro* study with human placenta (Radulovic *et al.*, 1986; 1987). Dearylation of methyl parathion through Cytochrome P450-mediated oxidation, dearylation of methyl paraoxon through hydrolysis by arylesterases (A-esterases or paraoxonase) and/or GSH-dependent aryltransferase all yield *p*-nitrophenol.

Comparing the GSH-dependent detoxification and microsomal activation, Rao and McKinley (1969) noted a generally higher activity of demethylation than activation in liver enzyme systems from rats, chickens, guinea pigs, and monkeys. In various experimental species, the *in vitro* methyl paraoxon demethylation catalyzed by GSH-transferases had considerably higher rates than the rate for either methyl paraoxon dearylation or methyl parathion demethylation (Benke and Murphy, 1975; Benke *et al.*, 1974; Hollingworth *et al.*, 1973; Radulovic *et al.*, 1987; Rao and McKinley, 1969). However, these *in vitro* studies will most likely not be predictive of the complex balance between hepatic detoxification and activation *in vivo* (Anderson *et al*., 1992; Huang and Sultatos, 1993). For example, studies with diethyl maleate and buthionine sulfoximine (GSH synthesis inhibitor) indicated that the contribution of GSH-dependent

Figure 1. Major Metabolic Pathways of Methyl Parathion.



biotransformation pathways might not be of great significance *in vivo* because of the preferential distribution of methyl parathion to the hepatocyte membranes in mice (Sultatos and Woods, 1988; Huang and Sultatos, 1993).

When given orally, methyl parathion is expected to undergo considerable first pass metabolism in the liver (Braeckman *et al.*, 1983; Sultatos, 1987). *In vivo* or *in situ* studies would therefore likely be more useful in providing integrated information on the metabolic pathways. Hepatic biotransformation of methyl parathion has been studied *in situ* in perfused livers of rats (Zhang and Sultatos, 1991) and mice (Sultatos, 1987). Based on the appearance of methyl paraoxon in the effluent perfusate, the net biotransformation of methyl parathion in the liver would appear to be activation.

3.4. Elimination

Studies in rats and mice showed that the metabolites of methyl parathion after oral exposures are almost exclusively eliminated in the urine (Hollingworth *et al.*, 1967; Menzie, 1969; Morgan *et al.*, 1977). The recovery in feces generally represented less than 10% of the administered dose (Miyamoto *et al.*, 1963a; Hollingworth *et al.*, 1967). Dimethyl phosphoric and phosphorothioic acid, desmethyl phosphate, desmethyl phosphorothioate, methyl phosphoric acid, phosphoric acid, and phosphate were identified in the urine of mice within 24 hours after oral administration of 3 or 17 mg/kg ^{32}P-methyl parathion (Hollingworth *et al.*, 1967). The relative proportion of these metabolites in the urine varied with doses.

In a study with four volunteers who were given 1 to 4 mg/kg methyl parathion orally, Morgan *et al.* (1977) reported that the overall elimination of methyl parathion was nearly complete in 24 hours. The amount of *p*-nitrophenol and dimethyl phosphate in the urine correlated well with the exposure to methyl parathion. The excretion rate of *p*-nitrophenol was rapid and nearly complete by the end of 8 hours of exposure. On the other hand, the rate for dimethyl phosphate was more prolonged and peaked at 4-8 hours after exposure (Morgan *et al.*, 1977).

4. BIOMONITORING

Urinary *p*-nitrophenol has been used extensively as an index of occupational exposure to various OP insecticides. The above study by Morgan *et al.* (1977) in 4 humans supported the use of urinary *p*-nitrophenol levels as a biomonitoring marker of exposure. The amount of urinary *p*-nitrophenol within 24 hours of exposure represented 7-29% (average 27%) recovery of the 2 or 4 mg/kg ingested methyl parathion. Based on the findings that recovery of *p*-nitrophenol peaked during the first 4 hours and was nearly complete by 8 hours, the authors emphasized the importance of urine sample collection from the beginning of exposure (Morgan *et al*., 1977). Chang *et al.* (1997) studied the correlation between the markers of toxicity and the urinary *p*-nitrophenol levels in Wistar rats that received 0 (corn oil), 1.4, 2.8, 5.6, or 7.0 mg/kg methyl parathion by gavage. The results showed good correlations between

methyl parathion exposures and both the plasma ChE inhibition and the level of urinary *p*-nitrophenol. The authors recommended using *p*-nitrophenol at 2.0 mg/g creatinine as the biological exposure index for methyl parathion.

In an effort to identify correlates for estimating the exposure resulting from indoor residential contamination scenarios, Estaban *et al*. (1996) analyzed the investigation records of the illegal indoor applications of methyl parathion during 1991 to 1994 in more than 200 homes in Lorain county, Ohio. The creatinine-adjusted *p*-nitrophenol level in the urine was positively correlated with the concentrations of methyl parathion in the air $r = 0.73$) and the residue on indoor contact surfaces $r = 0.48$). The median urinary *p*-nitrophenol level for children less than 3 years of age was approximately 5-fold higher than the adults. Further data and analysis are needed for quantitatively modeling the residential exposures based on the air and surface monitoring data. Meanwhile, USEPA issued guidelines in 1997 specifically for responding to methyl parathion contamination due to the illegal indoor applications. Urinary level of *p*-nitrophenol was established in the guidance as the prime determinant for temporary relocation of residents under the Superfund cleanup programs.

5. TOXICITY VARIATION AND MODIFICATION

Varying sensitivity to the toxicity of methyl parathion has been demonstrated in the available toxicity database with respect to species, age, and gender. *In vitro* and *in vivo* studies provided some biochemical basis for the variations. Metabolic modifiers and pharmaceutical chemicals have also been shown to alter the toxicity manifestation of methyl parathion. In addition, repeated exposure to OPs has been known to increase the tolerance for the effects of ChE inhibition. These aspects were considered in the selection of critical thresholds of toxicity and in the interspecies and inter-individual extrapolations of the subsequent risk assessment.

5.1. Interspecies and Inter-individual Sensitivity

Benke *et al*. (1974) compared the sensitivity of brain and muscle ChE in sunfish and in mice by incubating tissues with methyl paraoxon. The authors noted that ChE in sunfish was 10 to 100 times more resistant to inhibition by methyl paraoxon than the ChE in mice (see: Table 2).

Many of the major enzymes involved in the metabolic activation and detoxification of methyl parathion have been studied and compared *in vitro* with respect to species, sex, and age. Johnsen and Dahm (1966) reported greater interspecies variations in the capability for activation than for detoxification. Among rats, mice, guinea pigs, cattle, hogs, sheep, chicken, and ducks, the activation capability was the highest in rats (Johnsen and Dahm, 1966). With regard to gender, the capability in male rats was 23% higher than in the females. The greater activation capability may have contributed to the lower oral LD_{50} values reported for the male rats (see: Table 1). However, it should be noted that the higher

activation capability found in rats cannot account fortheir apparent higher sensitivity than other species to the activated form, methyl paraoxon (see: Table 2).

Paraoxonase, or A-esterase, dearylates and thereby detoxifies methyl paraoxon (Fig. 1). Although the liver possesses the detoxification mechanisms, paraoxonase (PON1) in the serum also plays an important role in the detoxification of OPs (Li *et al*., 1993; Costa and Manzo, 1995; Furlong *et al.*, 1998). Serum PON1 is a constituent of the high density lipoprotein (HDL). In addition to metabolizing xenobiotics, PON1 is also capable of hydrolyzing lipid peroxides and thereby is potentially useful in protecting against atherosclerosis by preventing the oxidation of low density lipoprotein (LDL) (Erdos and Boggs, 1961; Mackness *et al*., 1996; Mackness *et al*., 1997; Mackness *et al.*, 1998a). Spurred by these interests, much progress has been made recently in characterizing serum PON1 and its polymorphic distribution in human population.

Polymorphism in human population correlates to two pairs of amino acid substitution within the alleles of an autosomal locus on chromosome 7. One is a glutamine (Gln) ÷ arginine (Arg) substitution at position 192 (or 191, if Ala is defined as the N-terminal residue). The other is a methionine 6 leucine substitution at position 55 (Mackness *et al*., 1998b). With respect to its hydrolytic activity to paraoxon, the PON1 - 192 genotype was shown to correspond to the phenotype of homozygous low activity (Gln; QQ or AA type), homozygous high activity (Arg; RR or BB type) and heterozygous (QR or AB type) mid activity (Mackness *et al*., 1996). The PON1 - 55 methionine homozygous showed lower paraoxonase activities than the methionine-leucine heterozygous and leucine homozygous. Davies *et al*. (1996) demonstrated that the activities of PON1 isoforms were substrate specific. Within the 92 individuals of Hispanic origin, the *in vitro* serum PON1 activities of the homozygous Arg_{192} were 3-fold higher than the homozygous Gln_{192} when using paraoxon as the substrate. However, when using diazoxon, sarin, and soman as substrates, the results were reversed. The homozygous Gln_{192} showed higher hydrolytic activities (e.g., approximately 9-fold in the case of sarin) than the homozygous Arg_{192}. No significant difference in PON1 activities among the isoforms was noted when using chlorpyrifos oxon and phenylacetate as substrates.

Serum paraoxonase in humans shows a polymorphism with varying distribution patterns in different racial/genetic groups. Substantial differences in paraoxonase activities were also reported for individuals within a genetic group and a specific ethnic subpopulation. Geldmacher-von Mallinckrodt and Diepgen (1987) surveyed 37 European and non-European population groups for the distribution patterns of calcium ion-dependent paraoxonase activities. The results showed that approximately 53% of Europeans were in the low activity category. On the other hand, "Mongoloids" (denoted by the authors for Japanese, Vietnamese, Filipinos, Malayans, and Indonesians) and "Negroids" (denoted by the authors for Senegalese, Nigerians, Zambians, Zimbabweans, and Zulus) had much lower frequency or near absence of the low activity category. Overall data from Playfer *et al*. (1976) and Diepgen and Geldmacher-von Mallinckrodt (1987) showed an approximately 6-fold variation among the means of the three phenotypic categories, and as much as 40-fold variation among the individuals in a given

population. Costa and Monzo (1995) noted that the activity of paraoxonase could vary up to 13-fold among individuals homozygous for the low activities and up to an overall of 60-fold in humans.

The level of PON1 in newborn infants is approximately 50% of the adult level (Mackness *et al*., 1996). The level generally remains stable in an individual beyond the first year of life. This 2-fold age difference seems small compared to the much greater polymorphic variations. Lower PON1 activities were reported in Tangier disease (a rare heritable disease of lacking HDL) and systemic amyloidosis (build up of amyloid protein in tissues/organs) (Mackness *et al*., 1998a). On the other hand, higher PON1 activities were associated with inflammation and pyloric stenosis (narrowing of pyloric sphincter of the stomach) in infants before corrective surgery (Mackness *et al*., 1998a).

The significance of PON1 in the overall sensitivity to OP toxicity has been studied with a number of animal models. Species sensitivity to OPs was shown to be associated with paraoxonase activities. For example, the high susceptibility of birds to OPs correlates with low or non-detectable paraoxonase activities. Also, in mammals, the approximately 7-fold higher serum paraoxonase activity in rabbits than in rats corresponded to approximately 4-fold less sensitivity to paraoxon toxicity in rabbits (Costa *et al*., 1987). The impact of serum PON1 to the toxicity of OPs can also be demonstrated in knockout mice that lack PON1. In the studies reported by Furlong *et al*. (1998) and Shih *et al*. (1998), mice were dermally treated for 4 hours with chlorpyrifos oxon ranging from 1.5 to 15 mg/kg. Compared to the control mice, much greater AChE inhibition in the brain and diaphragm was reported in the knockout mice. For example, Furlong *et al*. (1998) reported that, while control mice showed no brain AChE inhibition and 70% diaphragm AChE inhibition at 7 mg/kg, the knockout mice had total inhibition of both brain and diaphragm AChE at 3.75 and 7 mg/kg, respectively. Death also occurred in knockout mice at 7 mg/kg after 2.5 hours of exposure. Similarly, Shih *et al*. (1998) reported at 6 mg/kg, only mild AChE inhibition in the brain and diaphragm of control mice while the AChE inhibitions were severe in the knockout mice, with clear cholinergic signs and death.

The use of serum paraoxonase status as a genetic marker for susceptibility has recently been suggested by Costa and Manzo (1995). Preliminary studies on Gulf War veterans also indicated the possibility of identifying individuals with high risk of OP poisoning based on PON1 markers (Markness *et al.*, 1998a). Given the role of paraoxonase in paraoxon detoxification, PON1 polymorphism would be expected to contribute to the susceptibility to methyl parathion. However, current data and consensus on the approach for a quantitative estimation on the range of human sensitivity specific to methyl parathion are unavailable.

Concerns have also been raised by the Scientific Review Panel (SRP), at the June 1999 deliberation under the California Toxic Air Contaminant Act, regarding the possible variation in sensitivity to OPs due to undernourishment. Changes in ChE levels associated with malnutrition have been reported. Dabke *et al* (1972) and Nagaraj *et* al (1981) reported a 50-60% lower serum BuChE among children and adults with severe protein and caloric malnutrition accompanied by 40-50% lower serum albumin

levels. Various degrees of recovery were noted after rehabilitation. Studies in rats also showed altered brain AChE levels with malnutrition occurring before weaning and during the postnatal brain growth spurt (3-5 weeks old). However, results were conflicting with respect to increased or decreased AChE activities. Noting the different timing of treatment in these studies, Wiggins *et al* (1984) concluded that brain AChE generally increased with continuing undernourishment or after nutritional rehabilitation. Singh *et al* (1990) reported a 39-46% brain AChE reduction in rat pups restricted from feeding for14 hours per day during postnatal day 5 to 20. The treatment resulted in an approximately 50% lower pup body weight. Reductions of AChE in four regions of brain (cerebral hemispheres, cerebellum, brainstem, diencephalon plus basal ganglia) were also reported in adult Wistar rats after 96 hours of food deprivation (Kaur and Kaur, 1992). Using ^{3}H-methyscopolamine, Viana *et al* (1997) reported a 21-26% reduction in muscarinic receptor binding in hippocampus and basal ganglia regions when rats were treated with diets deficient in protein and calories throughout gestation and continued to 3-5 months of age. The treatments resulted in 68% lower body weight with no change in brain AChE level. The implication of these reported changes of plasma and brain ChE and ChE under severe malnutrition is unclear with respect to the OP sensitivity in humans. Studies are needed to characterize the relationship between the degrees of under nourishment and the sensitivity to cholinergic toxicities of OPs.

5.2. Chemical Interactions

Metabolic modifiers and other pharmaceutical drugs have been shown to modify the acute toxicity of methyl parathion in rodents. The acute toxicity of methyl parathion was reduced by the intraperitoneal (i.p.) injection pre-treatment of piperonyl butoxide (Mirer *et al.*, 1977; Sultatos and Woods, 1988), phenobarbital (Sultatos, 1987), and cimetidine (Joshi and Thornburg, 1986) in mice and cimetidine in rats (Joshi and Thornburg, 1986). Piperonyl butoxide is known to inhibit the cytochrome-P450 system. Phenobarbital induces cytosolic glutathione-S-transferase. Cimetidine inhibits gastric secretion and is commonly used to treat peptic ulcers. Contrary to the reduction of toxicity by the aforementioned chemicals, the acute toxicity of methyl parathion in mice was potentiated by the i.p. pre-treatment of diethyl maleate (Mirer *et al.*, 1977; Sultatos and Woods, 1988). Diethyl maleate conjugates with glutathione and thereby reduces the pool of glutathione in the tissue. Conversely, in mice, acetaminophen (analgesic, antipyretic) administered via i.p. injection (Costa and Murphy, 1984) and buthionine sulfoximine (glutathione synthesis inhibitor) administered via drinking water (Sultatos and Woods, 1988) did not affect the toxicity of methyl parathion. Among some antibiotics and therapeutic drugs that reportedly reduced the acute toxicity of methyl parathion in rats were: xylazine (sedative), disopyramide (antiarhythmic), rifamycin, and gentamicin (antibiotics) (Youssef *et al.*, 1981, 1987). Information on the specific mechanisms of interaction, however, is not available.

5.3. Tolerance from Repeated Exposures

It has been widely recognized that both humans and laboratory animals develop tolerance to cholinergic toxicities after repeated exposures to OPs at sublethal levels (Costa *et al.*, 1982). In long term studies, mild cholinergic signs of toxicity initially present at the beginning of OP dosing either were lessened or disappeared after days or months of continuing exposures. When given pre-treatments at a lower dose level, animals were able to withstand a subsequent higher level of OP exposure which would have been toxic to naive animals with no prior exposures. Repeated dosing with increasing levels of OPs also reduced or eliminated the overt signs of toxicity which would otherwise be present in naive animals. In addition to tolerances toward OPs, animal studies also reported tolerance to the effects of direct acting cholinergic agonists (e.g., carbachol) or antagonists (e.g., atropine). Tolerance specifically to the toxicity of methyl parathion was quantitatively demonstrated in an early study by Galal *et al.* (1977) using 10 rats per group in a lethality study. The median lethal dose (LD_{50}) for the oral route was approximately 40% higher for animals that received increasing amount of methyl parathion over a 36-day period than for the naive animals. Several mechanisms for the tolerance have been proposed. To date, down-regulation of the muscarinic cholinergic receptors has been the mechanism with the most abundant support. It is important to note that while tolerance to OPs is well documented with respect to clinical signs, tolerance for several neurobehavioral and function effects has not been demonstrated (Llorens *et al*., 1993).

6. ACUTE TOXICITY

This section presents the toxicity database in humans and laboratory animals after acute exposures. Data in humans mainly consist of reports of poisoning cases, typically regarding clinical signs common to OP exposures. In addition to the immediate cholinergic crisis, intermediate and long term toxicity of acute OP exposures have also been noted. Only a general description of these effects is presented in this section while greater details are given for data specific to methyl parathion. The database from a substantial number of acute toxicity studies in laboratory animals is then presented. These included those studies that are required for fulfilling the registration purposes and those reported in the open literature. Specifically presented are the median lethal doses and concentrations for various routes of exposures, the patterns and levels of ChE inhibitions, and the influence of developmental age and maturity on the toxicity manifestation. Finally, detailed descriptions are given for studies that are pertinent for delineating the acute toxicity thresholds for characterizing the risk of human exposures to methyl parathion.

6.1. Effects in Humans

DPR maintains a record of the pesticide illness monitoring and investigative program in California. These records are published as annual reports of the *California Pesticide Illness Surveillance Program*. The circumstances of the exposure, however, often preclude a clear determination that the

reported clinical signs were exclusively due to exposures to methyl parathion. Cases of accidental and suicidal poisoning have also been reported in the open literature. In addition to the cholinergic crisis commonly documented for OPs, substantial attention in recent years has been devoted to investigating the delayed syndromes and chronic sequelae associated with an acute episode of poisoning. Most of these reports were concerned with exposures to OPs in general, while chemical-specific cases were too limited for differentiating any distinct patterns with a specific OP. Methyl parathion was specifically implicated in only a few cases. Other than an apparent pattern of being preceded by a cholinergic crisis, the biochemical mechanisms for these effects remain unclear.

6.1.1. Illness surveillance in California

Methyl parathion has been applied extensively in California for agricultural uses with no reported deaths attributable to its use. According to the most recent 3 years of available records on pesticide use in California (1993, 1994, 1995), approximately 140,000 to 175,000 pounds of methyl parathion was used annually in approximately 8,000 to 10,000 applications (DPR, 1995, 1996, 1997a). In California, 18 cases of illnesses from 1986 to 1995 were linked to methyl parathion (DPR, 1997b). These cases were rated as either "definitely", "probably", or "possibly" related to methyl parathion exposures, alone or in combination with other pesticides. Nine of the 12 cases of illness in 1990 were associated with rescue attempts after a midair collision of two crop dusters carrying a pesticide mixture of dimethoate, parathion, and methyl parathion. The majority of the remaining cases were associated with pesticide applications. Unfortunately, clinical signs were not recorded for the two cases identified as “definitely” associated with methyl parathion.

6.1.2. Cholinergic crisis

As with other OP pesticides, the signs of acute toxicity associated with methyl parathion poisoning are those characteristic of cholinergic abundance, such as, salivation, lacrimation, miosis, defecation, urination, headache, vertigo, respiratory distress, “twitching”, convulsions, and death due to respiratory failure (Murphy, 1986). Documentation of lethal oral exposure to methyl parathion in humans prior to 1976 were summarized in a NIOSH report (NIOSH, 1976). In one case, a 50 year old man died after reportedly drank an unspecified liquid with a total ingestion of 1.8 g of methyl parathion (approximately 25 mg/kg assuming a body weight of 70 kg). Most of the other reported deaths were associated with the ingestion of between 50 g to 300 g of methyl parathion. Results of autopsies from 30 fatal cases of human poisoning reported by Fazekas and coworkers between 1964 and 1969 (as cited in NIOSH, 1976) showed general edema and hemorrhage in liver, heart, spleen, kidney, brain, and gastrointestinal tract.

6.1.3. Intermediate syndrome

The intermediate syndrome (IMS) was mainly documented in OP poisoning cases from Sri Lanka, Belgium, Turkey, and India (Ecobichon, 1994; De Bleecker, 1995; Eyer, 1995; Padilla, 1995). These studies not only provided useful information for clinicians but also heightened the need to understand the mechanisms of acute toxicity to OPs. These syndromes appeared to be similar to the "Type II" symptoms of OP poisoning described earlier by Wadia *et al.* (1974), possibly nicotinic effects that became obvious after the successful treatment of muscarinic effects with atropine. The delayed neurological sequelae was more recently termed "intermediate" because they occurred after the initial stabilization of the acute cholinergic crisis and before the expected time for the onset of the well-characterized organophosphate induced delayed neuropathy (OPIDN) (Senanayake and Karalliedde, 1987). The latter subject is presented in Section 14, DELAYED NEUROPATHY.

Typically, IMS was noted 1 to 4 days after the successful treatment of cholinergic crisis. Patients developed myopathy, sudden respiratory paralysis, cranial motor nerve palsies, and weaknesses in proximal limb muscle and neck flexor (De Bleecker, 1995). With proper supportive care, particularly for the respiratory paralysis, patients recovered in time (a few days to several months). Recently, the paralysis of vocal cord was also noted in a 2 year old Tennessee boy presumably exposed to an unspecified OP (not specified as methyl parathion) used in a residential extermination treatment (Thompson and Stocks, 1997).

The mechanism of IMS is unknown. Several case reports indicated that treatments of atropine or oximes were not directly effective. On the other hand, based on a study of paraoxon and fenthion in rats and observations of human poisonings, De Bleecker (1995) suggested the involvement of the initial persistent AChE inhibition. All eight IMS patients investigated by De Bleecker (1995) had severe plasma and RBC ChE inhibitions. One current hypothesis is that the apparent neuromuscular junctional dysfunction may result from degenerative changes at the motor end plate region associated with the initial over abundance of ACh (Ecobichon, 1994; De Bleecker, 1995). The overall pattern of IMS showing apparent association with severe OP poisoning also emphasizes the importance of adequate initial treatments (Ecobichon, 1994).

In the 1995 review, De Bleecker mentioned that although IMS is not restricted to just a few OPs, fenthion, dimethoate, and methyl parathion were the OPs of high risk (De Bleecker, 1995). However, the literature search did not reveal any report of IMS due to the exposure to methyl parathion alone except the report of IMS in 6 out of the 7 cases of poisoning involving equal-weight mixture of parathion and methyl parathion (De Bleeker *et al*., 1992). De Bleeker *et al.* suspected that methyl parathion was the likely agent responsible for the observed IMS. This was based on the reasoning that IMS has not been reported in the many cases of parathion poisoning, nor had it occurred to another 5 cases of parathion poisoning concurrently investigated in the study. Although much remains unknown about IMS, its association with clinically significant OP poisonings appeared to be the most consistent

common factor among all cases of IMS. There has not been reports of IMS associated with asymptomatic OP exposures (Eyer, 1995).

6.1.4. Chronic neurological sequelae

In addition to the immediate acute cholinergic signs and symptoms and the subsequent IMS, chronic neurological sequelae has also been reported in humans after significant acute exposures. Available in the open literature are three epidemiological studies of workers who sought medical care from acute OP poisoning (Savage *et al*., 1988; Rosenstock *et al*., 1991; Steenland *et al*., 1994). None of the studies were specific to any single OP. These studies showed no correlation between the severity of neurological sequelae and the severity of poisoning. Nor did they show correlation to the time lapse between the poisoning and the investigation. Nevertheless, the coverage on neuropsychological and neurobehavioral effects of OPs in this document is warranted because of the widespread use of methyl parathion that presents a realistic probability of exposure. Methyl parathion has also been used in several of the investigations of possible mechanisms of neuropsychological and neurobehavioral toxicities and *in vivo* studies on neurotoxicity.

Savage *et al*. (1988) evaluated the residual neurological effects of 100 individuals (at least 16 years old at the time of evaluation) who had OP poisoning at least 3 months prior to the study (the average was 9 years between the poisoning and the start of case evaluation). Of the 100 subjects, 11 had more than one poisoning occasion, 96 through occupational exposures, and 54 reportedly caused by methyl parathion. The participants received physical, neurological and electroencephalographic examinations, and neuropsychological tests (Wechsler Adult Intelligence Scale, expanded Halstead-Reitan battery, 3 subsets of Peabody Individual Achievement Test, Minnesota Multiphasic Personality Inventory). Neurological tests showed significantly more cases of memory and mood abnormalities among the poisoned individuals. The neuropsychological tests showed lower scores in intellectual functioning, academic skills, abstraction and flexibility of thinking, and simple motor skills.

Similar chronic neurological sequelae were also reported in a study by Rosenstock *et al*. (1991) among 38 male agricultural workers (15-44 years old at the time of poisoning) in Nicaragua who had been admitted to the hospital for unintentional OP pesticide poisoning 10 to 34 months (the average was approximately 2 years) prior to the study. The participants were evaluated through a modified World Health Organization core battery and supplementary (6 of 11 subsets) tests, 6 standardized tests, and the Scandinavian questionnaire for self reporting central nervous system symptoms (e.g., difficulties in memory and concentration, headaches, fatigue, depression, irritability). These workers had significantly worse performances than the matched controls on 8 of the 12 neuropsychological tests for attention, memory, visual-motor, and motor functions. The poisoned group also was more likely to report symptoms consistent with central nervous systems involvement.

Evidence of neurological sequelae was further documented in a more recent study by Steenland *et al*. (1994). A battery of tests was administered among 128 men ($16 years old) in California who had at least one OP poisoning occasion and sought medical attention between 1982 and 1990. The pesticides specifically mentioned as the primary cause of poisoning were chlorpyrifos, diazinon, dimethoate, demeton or oxydemeton methyl, mevinphos, parathion, phosalone. There were 38 cases in which the primary cause was not specified. The participants were evaluated through neurophysical examination, 5 nerve conduction tests, 2 vibrotactile sensitivity tests, 10 neurobehavioral tests, and 1 postural sway test. Compared to a group of matched controls, the increased number of neurobehavioral tests showing worse performances was found to generally correlate with the indication of severity of past poisoning (e.g., hospital stay, loss of days at work).

Laboratory studies on the long-term neurobehavioral effects of a single poisoning episode by OPs are generally lacking and preliminary. George *et al*. (1993) conducted a study in rats exposed to methyl parathion. The Hebbs-William Maze (appetite-motivated task) test, administered 5 times per day, was used to identify the potential for neurobehavioral effects of methyl parathion. Groups of 6 Wistar rats were administered 15 mg/kg methyl parathion (the estimated LD_{50}) via gavage while atropine was given to alleviate the immediate effects of methyl parathion. Three weeks after the exposure, rats were subjected to a 5-day maze test after 2 days of learning. Rats exposed to methyl parathion took approximately 70% more time to complete the maze on day 1 of test and became comparable to the controls on day 3 of the test. The need for the extra 2 days of learning was indicative of a compromised ability of recent memory (George *et al*., 1993). The patterns within the 5 trials per day showed no effects on the immediate memory. The authors also reported the lack of neurobehavioral effects after 3 weeks of inhalation exposures (30 minutes spray of 500 mg methyl parathion in 10 ml water), presumably at a lower dose level. Unfortunately, the report lacked quantitative details for a thorough review.

6.2. Studies in Animals

Acute toxicity studies are parts of the battery of toxicity studies required for the registration of pesticides in California. These studies were conducted primarily for establishing the median lethal dose (LD_{50}) or concentration (LC_{50}) and determining the toxicity category of the technical grade and the formulations. Depending on the dose range used in the test, it may be possible to establish an acute NOEL (No-Observed-Effect Level) from these limited studies. In risk assessment, two terms have commonly been used in delineating the threshold dose for non-oncogenic effects. The NOEL is the experimentally determined highest dose at which no effects were observed. The term No-Observed-Adverse-Effect Level (NOAEL) is sometimes used to emphasize the adversity of the endpoints that formed the basis of the NOEL. As the definition of adversity of effects is sometimes subjective, no distinction between the NOEL and NOAEL is made in this document for characterizing the risk.

In addition, to the required studies, many studies available in the open literature also provided pertinent information for risk assessment considerations, specifically regarding the patterns of ChE inhibition and the extent of variation in sensitivity with respect of age.

6.2.1. Median lethal dose and toxicity category

The median lethal doses (LD_{50}) or median lethal concentrations (LC_{50}) are listed in Table 1 for methyl parathion and in Table 2 for methyl paraoxon. Methyl paraoxon is more acutely toxic than its parent compound. The magnitude of difference in toxicity may be estimated by comparing the LD_{50} values for methyl parathion and methyl paraoxon as determined from a given study having the same experimental protocol as well as route of exposure. The comparison revealed that methyl paraoxon is more potent by 6- to 8-fold in rats, 1.5-fold in mice, and 5-fold in guinea pigs. In general, the values of LD_{50} or LC_{50} of methyl parathion and methyl paraoxon are lower for rats than for other species. The overall database in Table 1 and 2 indicated that among the species tested, females appear to be either equally or less susceptible than males.

Based on the LD_{50} and LC_{50}, technical methyl parathion (80% pure) is a Category I oral toxicant (oral LD_{50} < 50 mg/kg), Category II inhalation toxicant (4-hour inhalation LC_{50} at or below 500 mg/m^3 but greater than 50 mg/m^3), Category IV eye irritant (caused conjunctival redness in rabbits that was cleared by 48 hours) and a category IV dermal irritant (caused erythema in rabbits that was cleared by 48 hours). Results of patch tests for contact dermatitis conducted among 200 subjects that were either non-, ex-, or current- agricultural workers, were negative with methyl parathion (Lisi *et al.*, 1986).

6.2.2. Cholinesterase inhibition

The inhibitions of ChE activity associated with acute methyl parathion exposure in various species are summarized in Table 3. Although the inhibition of ChE is extensively documented, in the absence of cholinergic signs of toxicity, the toxicological significance of the inhibition of ChE, especially the BuChE in the plasma, is not obvious. The effort to characterize any apparent correlation between the plasma and/or RBC ChE inhibitions and clinical signs has been complicated by the variety of protocols for ChE determination used by different investigators. Venkataraman *et al.* (1994) reported good correlations between RBC ChE and the intensity of clinical signs in adult female Wistar rats that received a single lethal level of methyl parathion at 7.5 mg/kg and without the treatment of either atropine or diazepam.

Table 1. Acute LD_{50} and LC_{50} values for methyl parathion.

Species	LD_{50} or LC_{50}			References
	Males	Females	M/F[a]	
Oral (mg/kg)				
Rat	14.0	24.0	---	Gaines, 1969
Rat	24.5	---	---	Miyamoto *et al.*, 1963b
Rat	6.0	---	---	Hirschelmann and Bekemeier, 1975
Rat	12.0	18.0	---	Newell and Dilley, 1978
Rat	11.1	16.0	---	Kronenberg *et al.*, 1978
Rat	4.0	6.3	---	Auletta, 1984a
Rat	2.9	3.2	---	WHO, 1984
Rat	10.8	9.3[b]	---	WHO, 1984
Rat	25.0[c]	62.0[c]	---	Cuthbert and Carr, 1986*
Rat	---	---	9.2	Galal *et al.*, 1977
Mouse	35.0	---	---	Hirschelmann and Bekemeier, 1975
Mouse	---	---	17.0	Miyamoto *et al.*, 1963b
Mouse	---	---	18.5	Kronenberg *et al.*, 1978
Mouse	---	---	23.0	NIOSH, 1987
Rabbit	10.0	19.4	---	WHO, 1984
Rabbit	---	---	420	NIOSH, 1987
Guinea Pig	417	---	---	Miyamoto *et al.*, 1963b
Guinea pig	---	---	1,270	NIOSH, 1987
Dog	---	---	90	Hirschelmann and Bekemeier, 1975
Inhalation (mg/m³)				
Rat (1-hr)	257	287	---	Newell and Dilley, 1978
Rat (1-hr)	200	---	---	USEPA, 1975
(4-hr)	120	---	---	USEPA, 1975
Rat (4-hr) (nose only)	---	---	135[c]	Greenough and McDonald, 1986*

(continued)

Table 1. Acute LD_{50} and LC_{50} values for methyl parathion (cont).

Species	LD_{50} or LC_{50}			References
	Males	Females	M/F[a]	
Inhalation (mg/m^3) (cont.)				
Rat (4-hr)	---	---	34	NIOSH, 1987
Mouse (4-hr)	---	---	120	NIOSH, 1987
Dermal (mg/kg)				
Rat	67	67	---	Gaines, 1969
Rat	110	120	---	Newell and Dilley, 1978
Rat	46	41	---	WHO, 1984
Rat	566[d]	---	---	Ortiz *et al.*, 1995
Rat	---	---	63	NIOSH, 1987
Mouse	---	---	1200[e]	Skinner and Kilgore, 1982
Rabbit	---	---	350-1180	Deichmann, 1950
Rabbit	---	---	300	NIOSH, 1987
Rabbit	---	---	2000	Auletta, 1984b
Intravenous (mg/kg)				
Rat	9.0	14.5	---	Newell and Dilley, 1978
Rat	4.1	---	---	Miyamoto *et al.*, 1963b
Mouse	---	---	9.8	NIOSH, 1987
Mouse	---	---	13	Miyamoto *et al.*, 1963b
Guinea pig	50	---	---	Miyamoto *et al.*, 1963b
Intraperitoneal (mg/kg)				
Rat, weanling	3.5	---	---	Brodeur and DuBois, 1963
adult	5.8	---	---	
Rat	---	7.0	---	DuBois and Kinoshita, 1968

(continued)

Table 1. Acute LD_{50} and LC_{50} values for methyl parathion (cont).

Species	LD_{50} or LC_{50}			References
	Males	Females	M/F[a]	
Intraperitoneal (mg/kg) (cont.)				
Rat				
1 day old	0.75	0.75	---	Benke and Murphy, 1975
12 day old	3.5	3.75	---	
23 day old	4.66	5.66	---	
37 day old	6.9	7.6	---	
60 day old	5.75	8.0	---	
Rat	---	---	3.5	NIOSH, 1987
Mouse	9.3	---	---	DuBois and Kinoshita, 1968
Mouse	11.0	---	---	Benke *et al.*, 1974
Mouse	8.2	---	---	Mirer *et al.*, 1977
Mouse	---	---	8.2	NIOSH, 1987
Sunfish ---	---	>2500		Benke *et al.*, 1974
Subcutaneous (mg/kg)				
Rat	---	---	6	NIOSH, 1987
Mouse	---	---	18	NIOSH, 1987
Rabbit	---	---	230	Deichman, 1950

* Studies acceptable for fulfilling the acute data requirement for pesticide registration.

a/ Values not sex-specific.

b/ The experimental animals were not fasted.

c/ Technical grade containing 80% methyl parathion. The mass median aerodynamic diameter for the inhalation study by Greenough and McDonald (1986) was 1.95-2.44 Fm.

d/ Formulation containing 50% methyl parathion, 47% xylene, and toluene, nitrophenol and nitrobenzene. The LD_{50} is adjusted to the amount of methyl parathion and is based on lethality within 72 hrs. Rat movements were restrained after the dermal application.

e/ Mice were exposed via both feet; a protocol generally results in lower toxicity when compared to treatment on shaved backs, presumably due to the greater cuticle depth (Skinner and Kilgore, 1982).

Table 2. Acute ID_{50} values for methyl paraoxon.

Species	LD_{50} or LC_{50}			References
	Males	Females	M/F[a]	
Oral (mg/kg)				
Rat	4.5	---	---	Miyamoto *et al.*, 1963b
Mouse	---	---	10.8	Miyamoto *et al.*, 1963b
Guinea Pig	83	---	---	Miyamoto *et al.*, 1963b
Intravenous (mg/kg)				
Rat	0.5	---	---	Miyamoto *et al.*, 1963b
Guinea Pig	2.2	---	---	Miyamoto *et al.*, 1963b
Intraperitoneal (mg/kg)				
Mouse	7.3	---	---	Benke *et al.*, 1974
Mouse	4.0	---	---	Mirer *et al.*, 1977
Sunfish	17.8	---	---	Benke *et al.*, 1974

a/ Values not sex-specific.

Table 3. Cholinesterase (ChE) inhibition after acute exposures to methyl parathion and methyl paraoxon.

Species; Sex	Dose or Exposure	Post Expo. Time	ChE Activity (% of control)		Reference
			METHYL PARATHION		
Oral (mg/kg)					
Rat (M)	11.7	1 hr.	Whole blood	41%	Newell and Dilley, 1978
Rat (F)	2.1	1 hr.	Plasma	50%	Murphy, 1980
	2.8	1 hr.	Brain	50%	
	2.5	1 hr.	Diaphragm	50%	
Rat (M)	5	6 hr.	Plasma	44%	Enan *et al.*, 1982
		24 hr.		43%	
		48 hr.		24%	
	5	6 hr.	Brain	86%	
		24 hr.		53%	
		48 hr.		59%	
Hen	100	2 days	Brain	15%	Ohkawa *et al.*, 1980
Inhalation (mg/m³)					
Rat (M)	264	1 hr.	Whole blood	41%	Newell and Dilley, 1978
Dermal (mg/kg)					
Rat (M)	110	1 hr.	Whole blood	16%	Newell and Dilley, 1978
	85	6 hr.	Whole blood	36%	
Rat (F)	85	6 hr.	Whole blood	43%	Newell and Dilley, 1978
Rat (F)	10.4	12 hr.	Plasma	50%	Murphy, 1980
	8.9	12 hr.	Brain	50%	
	6.9	12 hr.	Diaphragm	50%	
	9.4	12 hr.	Liver	50%	
Mouse (M)	950[a]	24 hr.	Plasma	50%	Skinner and Kilgore, 1982
	550[a]	24 hr	RBC	50%	

(continued)

Table 3. Cholinesterase (ChE) inhibition after acute exposures to methyl parathion or methyl paraoxon (cont.)

Species; Sex	Dose or Exposure	Post Expo. Time	ChE Activity (% of control)		Reference
			METHYL PARATHION (cont.)		
Intravenous (mg/kg)					
Rat (M)	6.6	1 hr.	Whole blood	24%	Newell and Dilley, 1978
Rat (M)	1.8	1 hr.	Plasma	50%	Miyamoto *et al.*, 1963b
	2.1	1 hr.	Brain	50%	
Guinea Pig	24.0 (M)	1 hr.	Plasma	50%	Miyamoto *et al.*, 1963b
	28.0	1 hr.	Brain	50%	
Dog	10.0	30 min.	Plasma	40%	Braeckman *et al.*, 1980
	30.0	30 min.	Plasma	25%	
Subcutaneous (mg/kg)					
Rats (M); 3 months old					
	7.6	4 hr.	Plasma	50%	Pope and Chakraborti,
	8.8	4 hr.	Brain	50%	1992
(M/F); 7 days old					
	0.9	4 hr.	Plasma	50%	
	1.0	4 hr.	Brain	50%	
Intraperitoneal (mg/kg)					
Sunfish 200		24 hr.	Brain	50%	Benke *et al.*, 1974
	600	24 hr.	Muscle	50%	

(continued)

Table 3. Cholinesterase (ChE) inhibition after acute exposures to methyl parathion or methyl paraoxon (cont.)

Species; Sex	Dose or Exposure	Post Expo. Time	ChE Activity (% of control)		Reference
			METHYL PARAOXON		
Intravenous (mg/kg)					
Rat (M)	0.4	1 hr.	Plasma	50%	Miyamoto *et al.*, 1963b
	0.3		Brain	50%	
Guinea Pig (M)	2.0	1 hr.	Plasma	50%	Miyamoto *et al.*, 1963b
	1.5		Brain	50%	
Intraperitoneal (mg/kg)					
Sunfish,	6.8	24 hr.	Brain	50%	Benke *et al.*, 1974
	11.5	24 hr.	Muscle	50%	

a/ Mice were exposed via both feet; a protocol which generally results in lower toxicity when compared to treatment on shaved backs, presumably due to the greater cuticle depth (Skinner and Kilgore, 1982).

6.2.3. Age-Related Sensitivity

Benke and Murphy (1975) studied the age-related differences in the oxidative activation and cleavage of methyl parathion in the liver homogenate from male and female Holtzman rats. The ratio of the oxidative detoxification to the activation of methyl parathion appeared to differ by age. The ratio ranged from approximately 0.4 for 1 day old rats to approximately 1.8 for 56-63 day old rats. The increase in detoxification with age was also reported for all other detoxification mechanisms and correlated well with the age-dependent increase in the LD_{50} (see: Table 1).

The extent of the age-related sensitivity to methyl parathion can be estimated by the differences in the LD_{50} and the ED_{50} for ChE inhibition in rats. The LD_{50} via intraperitoneal injection reported by Benke and Murphy (1975) (Table 1) showed as high as approximately 10-fold greater sensitivity in 1 day old neonates than in 37–60 day old Holtzman rats. The plasma and brain ChE inhibition ED_{50} from subcutaneous administration reported by Pope and Chakraborti (1992) (Table 3) showed an approximately 8- to 9-fold greater sensitivity in 7 day old neonates than in 3 months old Sprague-Dawley rats.

A greater than 2-fold difference in age-related sensitivity was also demonstrated in a subcutaneous dosing study by Pope *et al*. (1991). Greater than 79% of brain ChE inhibition was detected in 7 days old neonates at 7.8 mg/kg while a similar level of brain ChE inhibition was observed in 80-100 days old rats at 18 mg/kg. The authors noted, however, that the recovery of brain ChE level after exposure was faster in neonates than in the adults. Four days after the exposure, neonate brain ChE returned to approximately 80% of the controls while adult brain ChE remained at approximately 40% of the controls. The specific toxicities of methyl parathion resulting from *in utero* exposures are collectively presented in Section 12., *DEVELOPMENTAL TOXICITY*.

6.3. Thresholds for Acute Toxicity

The database for delineating a threshold for acute (one to several days) toxicity is very limited. One relatively cursory study in humans is available for estimating a threshold for acute toxicity based on ChE inhibitions. Several acute toxicity studies in rats were available. However, most of these studies were conducted for determining the median lethal dose (or concentration) using a high dose range and had limited toxicological observations. In these cases, the lowest test dose was the lowest-observed-effect level (LOELs) and a NOEL could not be directly established within the studies.

A determination of the critical acute NOEL for risk assessment, however, is not limited to the selected studies presented in this section. Toxicological studies designed for evaluating a specific type of toxicity (e.g., developmental toxicity) may also be useful for identifying acute NOELs based on findings reported shortly after the onset of exposure and/or endpoints that can potentially result from a single

(e..g, teratological effects) or short-term of exposure. Since neurotoxicity is the predominant effect of methyl parathion, the toxicity thresholds established from neurotoxicity studies (Section 13.) are particularly pertinent. The selection of the critical acute NOEL for characterizing the risk is presented in Section 18.1., *RISK ANALYSIS - Hazard Identification and Dose-Response Assessment*.

6.3.1. Studies in humans

Rider *et al.* (1969) conducted a study among prisoners to determine the RBC and plasma ChE inhibitions from the exposures to methyl parathion. Five subjects (body weights not given) received daily oral doses of methyl parathion in capsules, and two subjects receiving corn oil served as controls. Methyl parathion was given in increasing doses for 33 days, beginning at 1 mg/day. The dose was incrementally increased by 0.5 mg/day/subject starting on day 2 of the study and continuing through day 29 (15 mg/day/subject dosage). The dose was subsequently increased by 1 mg/day until a maximum of 19 mg/day/subject on day 33. Before the start and at the end of the study, each subject received a physical examination and had blood counts and clinical chemistry tests including urinalysis and prothrombin time determination. Plasma and RBC ChE levels were measured twice weekly throughout the pre-test (approximately 30-day), test, and post-test periods (unspecified). No signs of toxicity or changes in blood counts or clinical chemistry were detected among the test subjects. Although a 15% decrease in plasma ChE activity was noted at 11 mg/day (day 21), no depression of plasma ChE activity was noted in any of the subsequent higher dose levels (11.5 - 19 mg/day). Based on the lack of ChE inhibition at the final highest dose tested, the 19 mg/day could be considered the NOEL. Assuming a body weight of 70 kg, the dose at the NOEL was 0.27 mg/kg/day. A tolerance to methyl parathion acute toxicity might have developed over the 33 days of incremental dosing. Consequently, the NOEL may not represent a level that will not elicit a cholinergic response when it is given to an individual who has not previously been exposed to methyl parathion.

Rodnitzky *et al.* (1978) examined the neurobehavioral effects of methyl parathion in humans. Two males, ages 53 and 62 (body weight not reported), were given methyl parathion at 2 mg/day for 5 days and, after a 1 to 8 week rest interval, again at 4 mg/day for 5 days. Assuming a default body weight of 70 kg, the respective estimated doses at 2 and 4 mg/day were 0.029 and 0.057 mg/kg/day. No significant effects were noted for plasma and RBC ChE. The respective levels of plasma and ChE activities were 118 and 103% at 0.029 mg/kg/day and 120 and 95% at 0.057 mg/kg/day (see Table 4). Neither were there effects in several neurobehavioral tests (verbal recall, visual retention, information processing time, language, vigilance, proprioception, anxiety, and depression). Compared to the studies by Rider *et al.* (1969), this study had more elaborate toxicity evaluations; however, the dose level was much lower. The highest tested dose of 0.057 mg/kg/day could be considered a NOEL for adult humans, albeit with substantial uncertainties due to the small sample size and the unusual age range for test subjects.

6.3.2. Studies in animals

Galal *et al.*, (1977) conducted a study in which 5 rats/sex/dose were administered a single oral dosing of methyl parathion (50% pure, impurities unknown). The toxicity of Sevin and malathion were also included in this study. Not specifying the particular pesticide, the authors reported the following clinical signs: salivation, "shivering", chromodacryorrhea, exophthalmos, hyperreflexia, respiratory distress ("labored breathing", "respiratory convulsions"). Methyl parathion at 8.0 - 28.5 mg/kg was reported as the "fatal range" and 5.3 mg/kg as the "maximal tolerated dose". Based on the very brief report, it could be assumed that the 5.3 mg/kg dose level was the LOEL for this experiment.

In the study conducted by Auletta (1984a), groups of 5 male and female CD rats were exposed to a single oral dose of methyl parathion (purity not stated) at 1, 5, or 20 mg/kg. Clinical signs of toxicity such as nasal and oral discharge, wet rales, and apparent decrease in general activity were observed at 1 mg/kg (incidence not specified). Additional effects reported at 5 and 20 mg/kg were: mortality (5 of the 10 rats at 5 mg/kg and all rats at 20 mg/kg), tremors, ataxia, hypopnea, hypoactivity, and ocular discharge. An apparent deficiency in this study for establishing a NOEL and LOEL for clinical signs is that a control group was not included. However, the effects observed at 1 mg/kg are generally recognized as signs of cholinergic toxicity. Therefore, the dose of 1 mg/kg can be considered as a LOEL from this study.

A study by Cuthbert and Carr (1986) was accepted for filling the acute oral data requirement for pesticide registration. Groups of 5 male and female Sprague-Dawley rats were administered technical methyl parathion (80% pure) at 20, 30, or 40 mg/kg (males), or 40, 70, or 100 mg/kg (females) via gavage in a corn oil vehicle. One day after dosing, one rat died at 20 mg/kg. Hypokinesia, piloerection, soiled coat, and hemodacryorrhoea were observed in the remaining 4 males. The lowest dose of 20 mg/kg (or 16 mg/kg adjusted for 80% purity) can be considered as the LOEL from this study.

A study by Greenough and McDonald (1986) was accepted for filling the acute inhalation data requirement for pesticide registration. Groups of 5 male and female Sprague-Dawley rats were exposed to 0.108, 0.168, or 1.134 mg/l technical grade methyl parathion (80% pure) in the air for 4 hours. The respective mass median aerodynamic diameter and the geometric standard deviation (in parenthesis) were 1.95(2.00), 1.96(1.97), and 2.44(1.84) Fm. At least 86% of the particles were below 4.7 Fm. At 0.108 mg/l, two of the 5 males died within 2 hours of exposure. Clinical signs observed in the surviving male and female rats included: salivation, subdued and hunched appearance, tremors, prostration, depression and labored respiration, hypokinesia, and opacity in the eye. The 4-hour inhalation exposure at the lowest level of 0.108 mg/*l* technical grade methyl parathion (or 96 mg/m^3 methyl parathion) can be considered as the LOEL from this study. Using the default breathing rate of 0.96 m^3/kg/day (or 0.16 m^3/kg in 4 hours) for rats and a 100% inhalation absorption, the estimated dose at the LOEL was 15.4 mg/kg (96 mg/m^3 x 0.16 m^3/kg).

In a study by Schulz *et al*. (1990) that evaluated the behavioral effects in male Wistar rats, groups of 20 male Wistar rats were administered 0, 0.22, or 0.44 mg/kg/day methyl parathion (60% pure, impurities unknown) via gavage. The report did not indicate whether the doses given had been adjusted for the purity of the test material. Although the treatment period lasted 6 weeks, the report mentioned an increased mortality in the treatment groups (15% and 20% respectively at 0.22 and 0.44 mg/kg/day, compared to 5% in the controls) only during the first week of dosing. The results of behavioral tests after subchronic exposures were presented in Section 13., *Neurotoxicity*. The 0.22 mg/kg/day can be considered as an acute LOEL based on the increased mortality within the first week. This is the lowest acute LOEL among the five studies presented in this section. However, uncertainty exists regarding the use of this value in delineating a threshold dose for characterizing the risk of acute exposures to methyl parathion. One area of the uncertainty was the lack of information regarding the potential contribution of the impurities to the overall toxicity. Unfortunately, the studies by Galal *et al*. (1977) and Auletta (1984a) were also deficient in this regard. Another area of uncertainty was associated with the lack of information regarding the circumstances under which death of animals occurred. Although the methyl parathion-treated groups had higher mortality rates, death also occurred in the control groups (tap-water controls and "handled-only" controls). Moreover, all death appeared to occur only during the first week of dosing. The lack of detail in reporting the acute toxicity may have been because it was not the focal emphasis of the study.

7. SUBCHRONIC TOXICITY

Two human studies and several subchronic (14 days to 3 months) studies in rats, mice, and dogs were available for review and NOEL determination. Data on ChE inhibition, when available, are presented in Table 4. Included in this section is a brief summary of information on inhalation and dermal toxicities published in foreign languages and cited by the World Health Organization (WHO), USEPA, or Agency for Toxic Substances and Disease Registry (ATSDR).

7.1. Oral Studies in Humans

Rider and coworkers conducted two studies (Rider *et al*., 1970, 1971) with male prisoners on the effects of OPs on ChE inhibition. These studies differed from the 1969 study described under the Acute Toxicity section (Section 6.3.1., *Studies in humans*) in that fixed doses instead of increasing doses were administered. Five subjects received daily doses of methyl parathion in capsules at 22, 24, or 26 mg/day for 4 weeks (1970 study) or at 28 or 30 mg/day for 30 days (1971 study). The activities of plasma and RBC ChE were monitored twice weekly. These studies were reported only as abstracts (<200 words) for platform presentations in scientific meetings. Assuming a default body weight of 70 kg, the respective estimated doses for 22, 24, and 26 mg/day were 0.31, 0.34, and 0.37 mg/kg/day. No change in ChE was reported at 22 mg/day. At 24 mg/day (0.34 mg/kg/day), the mean plasma and RBC ChE inhibitions for the 5 subjects were 17 and 22%, respectively. However, only two of the 5 subjects showed ChE inhibitions. The subject having a greater effect had 23 and 55% inhibition on

Table 4. Cholinesterase (ChE) activity following subchronic oral exposure to methyl parathion.

			ChE (% of control)						
	Dose[a]		Males			Females			
Sp.	mg/kg/day	Time	Plasma	RBC	Brain	Plasma	RBC	Brain	Reference
Humans									
n=5	0.31	4 wk	NA[b]	NA[b]	-	-	-	-	Rider *et al.*,
	0.34		77[c]	45[c]					1970
	0.37		NA[b]	63[c]	-				
n=5	0.40	30 d	-	(81)[d]	-	-	-	-	Rider *et al.*,
	0.43		-	(63)[d]	-				1971
n=2	0.029	5 d[e]	118	103	-	-	-	-	Rodnitzky
	0.057		120	95	-				*et al.*, 1978
Rats									
n=10	0.2	1 mo	118	70*	-	112	97	-	Daly and
	1.9		109	57**	-	71**	67*	-	Rinehart,
	5.7		82*	67*	-	46**	69	-	1980a
	0.2	3 mo	92	93	110	107	104	99	
	1.9		77	85	95	61**	88	68*	
	5.7		77	80	26**	32**	75	35**	
Mice		4 wk							
n=10									
27.3(M),25.5(F)			35**	86**	-	40**	86**	-	Eiben, 1988a
56.5(M),37.0(F)			39**	86**	-	24**	84**	-	
		65 d							
27.3(M),25.5(F)			37**	90**	33**	26**	88**	39**	
56.5(M),37.0(F)			23	82	33	16**	90**	42**	
n=10	0.93	4 wk	98	89**	-	96	92	-	Eiben, 1988b
	3.82		81**	85**	-	75**	84*	-	
	14.96		23**	80**	-	36**	82*	-	
	0.93	66 d	90*	100	92	92**	98	98	
	3.82		78**	92*	92	75**	93*	100	
	14.96		36**	82**	70**	42**	91**	80**	

(continued)

Table 4. Cholinesterase (ChE) activity following subchronic oral exposure to methyl parathion (cont.).

			ChE (% of control)						
	Dose[a]		Males			Females			
Sp.	mg/kg/day	Time	Plasma	RBC	Brain	Plasma	RBC	Brain	Reference
Dogs									
n=4	0.3	6 wk	87	79	-	93	94	-	Underwood
	1.0		71	67	-	80	82	-	and Tegeris,
	3.0		53*	23*	-	42*	34*	-	1978
	0.3	3 mo	86	80	113	92	87	114	
	1.0		72*	63*	98	84	64*	98	
	3.0		45*	27*	36*	37*	25*	44*	
n=8[f]	0.03	6 wk	82	100	-	96	100	-	Daly, 1989
	0.30		86	98	-	92	98	-	
	3.0		46**	78*	-	41**	80**	-	
	0.03	13 wk	81*	100	92	98	100	92	
	0.30		86	98	100	94	91	100	
	3.0		53**	82	46**	47**	77**	50**	

Levels of statistical significance as compared to the controls: * for p#0.05; ** for p#0.01. The report by Underwood and Tegeris (1978) provided statistical significance only for p#0.05.

a/ The concentration in the diet was either given in the report or converted to mg/kg/day based on food intake data.

I) Dose levels per body weight in human studies were estimated based on a default body weight of 70 kg for an adult. The corresponding dosing levels were 22, 24 and 26 mg/day for the study by Rider *et al.* (1970), 28 and 30 mg/day for the study by Rider *et al.* (1971), and 2 and 4 mg/day for the study by Rodnitzky *et al.* (1978).

ii) Dose levels for the study by Daly and Rinehart (1980a) corresponded to 2.5, 25, and 75 ppm in the diet.

b/ NA, not available. In an abstract for a platform presentation, the authors stated as "produced no effects" or "not significantly altered".

c/ Represented the lower of the 2 subjects who showed ChE inhibition.

d/ The ChE data for individuals was not given in an abstract for a platform presentation. Values given in the parenthesis represented the average of 5 subjects, some of which reportedly had no effects.

e/ The 5 day treatment period was repeated after 1 to 8 week rest interval.
Data of 2 subjects.

f/ Data on brain ChE was from the pons, with n=4. No ChE effect was noted in the cerebellum.

plasma and RBC ChE (Table 4). Based on the ChE inhibition, the subchronic NOEL was 22 mg/day (0.31 mg/kg/day). The NOEL of 0.31 mg/kg/day was used by the USEPA in setting a 10-day drinking water Health Advisory of 0.3 mg/*l*. This was calculated based on a daily drinking water consumption of 1 liter for a 10-kg child and applying an uncertainty factor of 10 to account for the inter-individual variations in sensitivity (USEPA, 1988).

In addition to the endpoints of ChE inhibition, Rodnitzky *et al*. (1978) examined the neurobehavioral effects in two human test subjects after 5 days of methyl parathion ingestion. The treatment was repeated after 1 to 8 weeks rest intervals. This study was previously described in Section 6.3.1. No effects were noted in this study, however, the dose range was much lower than the studies by Rider *et al.* described above.

7.2. Oral Studies in Rats

In the study by Galal *et al*. (1977) described in Section 6.3., *Thresholds for Acute Toxicity*, three groups of albino rats (5/sex/group) were initially administered methyl parathion at 0.37 mg/kg/day, an equivalence of 4% of the acute oral LD_{50} (9.2 mg/kg) determined from the same study. During the 36 days of dosing, the doses were successively increased by a magnitude of 1.5 geometric progressions (a stepwise dose increase by a factor of 1.5) every fourth day for each group. According to the description on dose increment, the final dose level would be 32 mg/kg/day. Changes in hematological parameters were noted at the end of the study. These included a decrease in RBC counts (approximately 45%), changes in differential leukocytic counts (increases in neutrophils and lymphocytes and decreases in monocytes and eosinophils), and an increase in coagulation time (up to 36%). The studies demonstrated a tolerance to the cholinergic toxicity of methyl parathion with the successive increase in dose (see: Section 5.3., *Tolerance after Repeated Exposure*). The pattern of dosing precluded the determination of a NOEL or LOEL from this study.

In a 1979 range-finding study by the National Cancer Institute (NCI, 1979), groups of 5 male and female Fischer F344 rats were fed diets containing 0, 10, 20, 30, 40, or 50 ppm methyl parathion (94.6% pure) for 7 weeks followed by a one week observation period. The survival and body weights were reported. All males survived the treatments. The respective survival for the females from the controls to the high dose group were 5/5, 4/5, 5/5, 4/5, 4/5, and 3/5. The microscopic examinations were reported as "essentially normal" at 30 to 50 ppm. Approximately 10% body weight decrease was noted at 40 and 50 ppm. Based on the one death at the lowest tested dose, the LOEL was 10 ppm. Using a default assumption that the food intake is approximately 5% of body weight, the estimated dose at this dietary level was 0.5 mg/kg/day.

A 3-month feeding study conducted by Daly and Rinehart (1980a) is on file at DPR. Groups of 20 Sprague-Dawley rats per sex were fed diets containing 0, 2.5, 25, or 75 ppm methyl parathion (93.65% pure). Based on food consumption data, the respective estimated doses were 0.2, 1.9, and 5.7 mg/kg/day. At 25 ppm, the respective activities of plasma, RBC, and brain ChE were 61-77%,

85 - 88% and 68-95% of the controls (Table 4). Additional treatment-related changes were noted at 75 ppm (5.7 mg/kg/day). These included decreases in: RBC count (12%, females only), hemoglobin (5-12%), hematocrit (9%, females), and blood glucose (15-23%); increases in alkaline phosphatase (36-84%) and blood urea nitrogen (22%, females only); decreases in body weight gain (20-24%) despite higher food consumption (25-29%); as well as staining of the ano-genital area, tremors, and emaciation. At week 4, one male and 14 females either died or were sacrificed moribund. Gastritis, lymphoid depletion and necrosis (lymph nodes, spleen, and thymus), necrosis of the submaxillary salivary glands, and bone marrow hypocellularity were observed mainly in animals that died during the first four weeks of the study. Based on the plasma, RBC, and brain ChE inhibition (as much as 32% reduction in brain ChE) the NOEL was 2.5 ppm in the diet (0.2 mg/kg/day). Although the RBC ChE inhibition in the male rats was 30% and statistically significant at 1 month, the lack of the same low level of inhibition cast some uncertainties to its pertinence. Based on endpoints other than ChE inhibitions (hematological indices, clinical chemistry, body weight gain reduction, ano-genital stain, tremors, emaciation, and death), the NOEL was 25 ppm in the diet (1.9 mg/kg/day).

In a study by Yamamoto *et al*. (1982), male Wistar rats were orally administered methyl parathion (98.8% pure), at 0.5, 1.5, 3.0, or 5 Fmol per rat (body weight of approximately 100 g), for as long as 10 days. Unspecified cholinergic signs of toxicity and slightly lower body weight (approximately 95% of the controls, estimated from the figure presented in the report) were reported at 0.5 Fmol (1.3 mg/kg/day). Methyl parathion treatments at the two higher doses (1.5 and 3.0 Fmol) resulted in the death of all the rats by day four of dosing. The plasma and brain ChE were respectively reduced to 70 and 43% of the controls after a single dosing at 5 Fmol/rat; and 76 and 57% after 10 days of dosing at 0.5 Fmol/rat. No toxicity observations other than death were included in the report. Based on the brain ChE inhibition, the lowest dose of 0.5 Fmol (1.3 mg/kg/day) was the LOEL.

7.3. Oral Studies in Mice

In a 1979 range-finding study by the NCI (1979), groups of 5 male and female B6C3F1 mice were fed diets containing 0, 20, 40, 60, 125, 250 or 500 ppm methyl parathion (94.6% pure) for 7 weeks followed by a one week observation period. The survival and body weights were reported. Except for one male at 20 ppm, the remaining males and females survived the treatments up to 125 ppm. The microscopic examinations were reported as “essentially normal” at 125 ppm. Approximately 10% body weight decrease was noted at 40 and 60 ppm. Based on the one death at the lowest tested dose, the LOEL was at 20 ppm. The estimated dose at this dietary level was 2 mg/kg/day.

Four studies are on file at DPR. A pilot study for a 90-day study was conducted by Daly and Rinehart (1979). Groups of 5 CD-1 mice per sex were fed diets containing 0, 25, or 50 ppm (93.65% pure)

for 29 days. The respective average doses over the study period were 0, 5.5, and 12 mg/kg/day. No effects were noted in daily physical examinations and postmortem gross necropsy. The only effect reported was a significant body weight reduction at 50 ppm. In the subsequent 3-month study (Daly and Rinehart, 1980b), groups of 15 CD-1 mice per sex were fed diets containing 0, 10, 30, or 60 ppm methyl parathion (93.65% pure). The respective doses were 0, 2.4, 7.6, and 15 mg/kg/day. As in the pilot study, no death, treatment-related clinical signs, treatment-related gross or microscopic changes or lesions were observed. Body weights were reduced by 4-20% in the males and 4-11% in the females at 60 ppm, although food consumption was increased (3-20% in the males and 4-23% in the females). The relative brain weight was increased by 3-10%. Some indices which may be more sensitive, such as ChE, hematology, serum chemistry or urinalysis, were not measured.

Eiben (1988a, b) conducted two 65-66 day range-finding studies in mice. Groups of 10 B6C3F1 mice per sex were fed diets containing methyl parathion (96.8% pure) at either 0, 50, or 75 ppm (Eiben, 1988a) or 0, 2, 8, 32, 128, or 400 ppm (Eiben, 1988b). The respective doses at 50 and 75 ppm were substantially different for the males and females; 27.3 and 56.5 mg/kg/day for the males and 25.5 and 37.0 mg/kg/day for the females. The doses for the second study by Eiben (1988b) were similar between the males and females; the average male and female doses at 2, 8, and 32 ppm were 0.93, 3.82, and 14.91 mg/kg/day. At 2 ppm (0.93 mg/kg/day), statistically significant ($p<0.01$) inhibition was noted for RBC and plasma ChE (Table 4). Brain ChE inhibition (23-30%) was significant at 32 ppm. Mice at 50 ppm or higher had been reported as having poor general condition, tremors, rough coats, sunken flanks, and loss of weight. Plasma cholesterol level was significantly elevated in the females at 50 and 75 ppm. Based on the brain ChE inhibition, the NOEL was 8 ppm (3.82 mg/kg/day). However, the lowest tested dose of 2 ppm (0.93 mg/kg/day) was the LOEL based on statistically significant ChE inhibitions of the plasma (male and female rats) and the RBC (male rats).

7.4. Oral Studies in Dogs

Williams *et al*. (1959) conducted a study in which dogs were fed diets containing 5, 20, or 50 ppm methyl parathion for 12 weeks. Plasma and RBC ChE inhibitions reached approximately 25-35% at 20 ppm and 45% at 50 ppm.

One 14-day and two 3-month feeding studies are on file at DPR. In the 14-day range-finding study (Underwood and Tegeris, 1977), groups of two dogs per sex received nominal doses of 0, 2.5, 5.0, or 10 mg/kg/day methyl parathion (94.3% pure) in the diet. The authors reported that vomiting, a possible cholinergic sign of toxicity, occurred in dogs (number not specified) at the high dose starting on the third day. Vomiting also occurred in all dogs at the mid-dose and one dog at the low dose, particularly during the second week of dosing. Food consumption and body weight gain were decreased in the mid- and high doses. Based on vomiting and decreases in body weight gain, the lowest test dose of 2.5 mg/kg/day was the LOEL for this study.

In the subsequent study by Underwood and Tegeris (1978), groups of 4 dogs per sex were fed methyl parathion (94.3% pure) in the diet at 0, 0.3, 1, or 3 mg/kg/day for 3 months. No treatment-related effects on mortality, food consumption, body weight gain, hematology, and gross or microscopic observations were noted. Other than occasional (undefined) vomiting in some test animals (at unspecified dose), the only effect noted was ChE inhibition. The activities of plasma and RBC ChE at the mid- and high dose, and brain ChE at the high dose were decreased (Table 4). Plasma and RBC ChE activities in male and female dogs at 1 mg/kg/day were respectively reduced to 72-84% and 63-64% of controls at the end of the treatment period. The NOEL was 0.3 mg/kg/day based on plasma and RBC ChE inhibition. The NOEL was 1 mg/kg/day based on brain ChE inhibition (at 36-44% of the controls at 3 mg/kg/day). The NOEL of 0.3 mg/kg/day was used by the USEPA in setting a long-term drinking water Health Advisory of 0.1 mg/*l* (USEPA, 1998). This was calculated based on a daily drinking water consumption of 2 liters for a 70-kg adult and applying an uncertainty factor of 100 to account for the interspecies and inter-individual variations in sensitivity.

In a latter study by Daly (1989), groups of 8 beagle dogs per sex were fed methyl parathion (94.9% pure) in the diet at 0, 0.03, 0.30, or 3.0 mg/kg/day for 13 weeks. At the end of the dosing period, 4 dogs per sex per group were sacrificed while the remainders were given a 4 to 6 week recovery period before sacrifice. At 3.0 mg/kg/day, the activities of plasma and RBC ChE were depressed at six and 13 weeks and brain ChE at 13 weeks (Table 4). The ChE activities returned to their baseline levels after the recovery period. Intraocular pressure was decreased in the mid-dose females and high dose males after the recovery period but not at the end of the 13-week dosing period. Anti-ChE agents are known to reduce the intraocular pressure and have been used in the management of glaucoma (Taylor, 1985; Gallo and Lawryk, 1991). The toxicological significance of this effect was not clear, especially also considering the lack of a demonstrated dose-response relationship. Electroretinograms and ophthalmoscopic and microscopic examination of the eyes did not show any remarkable changes. Two dogs at 3.0 mg/kg/day showed dehydration, emaciation and a thin appearance. One low-dose male continued to have a thin appearance throughout the recovery period. Based on the brain ChE inhibition (50-53%), the NOEL was 0.3 mg/kg/day. It is important to note that at the lowest dose of 0.03 mg/kg/day the level of plasma ChE inhibition in the males was consistent at both time points of measurement and was statistically significant at week 13. The NOEL for the eye effects would be 0.03 mg/kg/day.

7.5. Dermal Toxicity Studies

The toxicity of repeated dermal applications was reported in a study by Dikshith *et al*. (1991). Groups of 10 female albino rats received 2 mg/kg/day methyl parathion (50 EC, impurities unspecified) to the shaved latero-abdominal areas (10% of body surface area) for 7, 15, or 30 days. Four rats died by the end of 30 days. Other effects included reduced relative liver weight after 7 days of treatment (but not at 15 and 30 days of treatment), and degenerative changes reportedly occurred in the liver, kidney, and

brain. Brain and RBC ChE activities were inhibited by 35 and 71% respectively after 30 days of treatment. Based on the effects in the liver, kidney, and brain, the 2 mg/kg/day was the LOEL However, the lack of detail and possible inconsistencies in the reporting prevents further evaluation of this study. It is interesting to note that the dose of 2 mg/kg/day that resulted in 40% mortality was 23- to 283-fold lower than the LD_{50} values reported for acute exposures (Table 1).

7.6. Data from Reviews and Summaries

Information on methyl parathion toxicity through inhalation and dermal exposures is largely available only as summarized by World Health Organization (WHO, 1984), USEPA (1984), and Agency for Toxic Substances and Disease Registry (ATSDR, 1992). Many of the citations were in foreign languages. No deaths or treatment-related histopathological lesions were reported in a study by Thyssen and Mohr (1982; as cited in WHO, 1984) in which, groups of 10 rats per sex were exposed to 0, 0.9, 2.6, or 9.7 mg/m³ methyl parathion, 6 hours/day and 5 days/week, for 3 weeks. Rats at the high dose had significant plasma and brain ChE inhibition (levels not specified), body and organ weight reduction, and cholinergic signs. Slight inhibition of plasma ChE was noted at 2.6 mg/m³. The apparent NOEL was 2.6 mg/m³ (estimated dose of 0.45 mg/kg/day) based on clinical signs of toxicity.

Toxicity associated with dermal exposure to methyl parathion was reported by Mihail and Vogel (1984; as cited by WHO, 1984). Groups of 6 rabbits per sex received un-occluded topical applications of 0, 50, or 250 mg/kg/day methyl parathion (in Cremophor E.I.), 6 hours/day and 5 days/week, for 3 weeks. Epithelial proliferation with hyperkeratosis was noted at the treatment site. Methyl parathion treatments had no effects on hematology, serum chemistry, and organ weights. Activities of RBC and brain ChE were inhibited in both dose groups in a dose-related manner. Cholinergic signs, deaths, and significant inhibition in plasma ChE (level not specified) also occurred at 250 mg/kg/day. A subsequent study was conducted using 10 mg/kg/day in an effort to establish a NOEL. No ChE inhibition or dermal effects were reported at 10 mg/kg/day.

The USEPA (1984) documented several oral exposure studies that were reported in foreign journal publications. Rats that received methyl parathion at 0.15 or 0.75 mg/kg/day for 1, 2 or 4 months had depressed renal tubule succinic dehydrogenase, increased glomerular alkaline acid phosphatase, "morphological changes in myocardial and scar tissues, focal granuloma, vascular sclerosis, bronchitis, stratification of blood vessels, hyperplasia of pulmonary tissue, and lymphatic atrophy" (Zlateva and coworkers, 1977, 1978; as cited in USEPA, 1984). Delayed conditioned reflexes to light and sound were reported in rats that received 30 Fg/kg/day (0.03 mg/kg/day) methyl parathion in drinking water for 6-9 months (Cabejszek and Szulinski, 1966; as cited in USEPA, 1984). Decreased white blood cell phagocytic activity, complement titer, serum lysozyme, and nucleic acid content in blood occurred in rabbits that received methyl parathion orally at 5 mg/kg/day (in sunflower oil), 6 days/week for 4 months (Samedov *et al.*, 1979; as cited by USEPA, 1984).

8. CHRONIC TOXICITY

The toxicity of methyl parathion after chronic (beyond 1 year) exposures has been studied in rats, mice, and dogs. Data on ChE inhibition, when available, are presented in Table 5. Significant brain ChE inhibition was consistently reported in these studies. Among other neurological effects was myelin degeneration reported in a 12 month study in rats.

8.1. Oral Studies in Rats

A total of 4 studies were included in this section. The studies by Bomhard *et al*. (1981), Daly and Hogan (1983), and Daly (1991) are on file at DPR. A study by Nagymajtenyi *et al*. (1995) is available in the open literature. In the study conducted by Bomhard *et al*. (1981), groups of 50 Wistar rats per sex were fed diets containing 2, 10, or 50 ppm methyl parathion (94.8% pure) for 2 years. The controls consisted of 100 rats per group. Interim sacrifices were performed on additional groups of five animals at 6 and 12 months after the start of the study. There was an approximately 15% increase in food consumption in the females at 50 ppm. However, the body weight of the males and females at 50 ppm was approximately 9% lower than the controls. Based on the food intake data, the corresponding methyl parathion intake was 0.09, 0.46, and 2.6 mg/kg/day for the males and 0.14, 0.71, and 5.0 mg/kg/day for the females. Cumulative mortality data for the first six months showed a statistically significant increase in the females at 50 ppm (16% mortality). Transient alterations in hemoglobin, hematocrit, and reticulocyte counts, as well as decreased plasma protein levels, and increased levels of plasma urea and protein in the urine, were noted at 50 ppm throughout the exposure period. These indices were indicative of liver and kidney injury. The activities of plasma and RBC ChE were significantly reduced at 10 and 50 ppm from as early as week 2 and throughout the treatment period.

Moreover, the RBC ChE inhibition at 2 ppm was statistically significant at many measurement time intervals. Brain ChE inhibition was detected at the end of the study at 10 and 50 ppm (Table 5). The NOEL for brain ChE inhibition was 2 ppm (0.09 mg/kg/day). Based on statistical significance, the lowest dose of 2 ppm was the LOEL for the RBC ChE inhibition. It should be noted that clinical observations were not reported for all animals. Also, no histopathological examinations of the spinal cord and peripheral nerves were performed. Due to these and other deficiencies (e.g., lacking descriptions of diet preparation and test substance analyses in the diets) this study was judged unacceptable to DPR for filling the SB950 chronic toxicity data requirement based on the FIFRA study guidelines.

A study by Daly and Hogan (1983) was acceptable under FIFRA guidelines for filling the data requirements for chronic and oncogenicity studies in rats. Groups of 60 Sprague-Dawley CD rats per sex were fed diets containing 0, 0.5, 5.0, or 50 ppm methyl parathion (93.7% pure) for 25 months (males) or 28 months (females). Five animals per group were killed at 24 months as an

Table 5. Cholinesterase (ChE) activity following chronic dietary exposure to methyl parathion[a].

Sp.	Dose or Conc. in feed	Duration	ChE (% of control) Males Plasma	Males RBC	Males Brain	Females Plasma	Females RBC	Females Brain	Reference
Rats									
n=5	2 ppm	2 wk	91	96*	-	95	93	-	Bomhard
	10 ppm		73**	84**	-	78	76**	-	*et al.*, 1981
	50 ppm		36**	67**	-	21**	67**	-	
	2 ppm	13 wk	85	93*	-	86	80**	-	
	10 ppm		80	73**	-	61*	73**	-	
	50 ppm		36**	66**	-	14**	58**	-	
	2 ppm	26 wk	88	94	-	95	90*	-	
	10 ppm		82	84**	-	69	78**	-	
	50 ppm		61*	82**	-	19**	68**	-	
	2 ppm	52 wk	92	83**	-	89	84**	-	
	10 ppm		73*	75**	-	75	79**	-	
	50 ppm		32**	69**	-	12	71**	-	
	2 ppm	105 wk	108	94	89	104	94	102	
	10 ppm		111	78**	78*	73	78**	106	
	50 ppm		41	68**	50**	13**	65**	37**	
n=10	0.5 ppm	26 wk	86	95	-	100	107	-	Daly and
	5 ppm		71	91*	-	90	104	-	Hogan, 1983
	50 ppm		29**	87**	-	14**	100	-	
	0.5 ppm	52 wk	89	100	-	100	101	-	
	5 ppm		100	99	-	84	99	-	
	50 ppm		33**	84*	-	13**	92*	-	
	0.5 ppm	104 wk	109	102	106	100	96	95	
	5 ppm		91	96	99	106	89	98	
	50 ppm		18**	91**	24**	18**	95	21**	

(continued)

Table 5. Cholinesterase (ChE) activity following chronic dietary exposure to methyl parathion (cont.).

Sp.	Dose or Conc. in feed	Duration	ChE (% of control) Males Plasma	Males RBC	Males Brain	Females Plasma	Females RBC	Females Brain	Reference
Rats									
n=10[b]	0.5 ppm	1 mo	90	93	-	105	103	-	Daly, 1991
	2.5 ppm		101	92	-	81	103	-	
	12.5 ppm		75*	87*	-	67	90*	-	
	50 ppm		48**	80**	-	26**	86**	-	
	0.5 ppm	6 mo	83	101	-	112	103	-	
	2.5 ppm		86	97	-	84	102	-	
	12.5 ppm		65	94	-	72	91	-	
	50 ppm		39**	87**	-	21**	87**	-	
	0.5 ppm	52 wk	91	99	95	105	97	93	
	2.5 ppm		103	96	96	90	93	99	
	12.5 ppm		71	97	96	67*	88**	75**	
	50 ppm		37**	86*	43**	30**	80**	25**	
Mice									
n=10	1 ppm	52 wk	113	92	87	100	103	100	Eiben, 1991
	7 ppm		94	43*	63*	94	51*	99	
	50 ppm		29*	14*	16*	35*	24*	54*	
	1 ppm	104 wk	86	93	81	109	90	97	
	7 ppm		104	48*	77*	104	59*	92	
	50 ppm		25*	11*	33*	39*	20*	38*	
Dogs									
n=8		2 mo							Ahmed and
	0.03 mg/kg/day		99	97	-	100	95	-	Sagartz, 1981
	0.1 mg/kg/day		108	86*	-	107	86	-	
	0.3 mg/kg/day		83*	82*	-	87	65	-	
		4 mo							
	0.03 mg/kg/day		99	97	-	81*	93	-	
	0.1 mg/kg/day	110	90	-	72*	93	-		
	0.3 mg/kg/day	84*	80	-	52*	81	-		

(continued)

Table 5. Cholinesterase (ChE) activity following chronic dietary exposure to methyl parathion (cont.).

Sp.	Dose or Conc. in feed	Duration	ChE (% of control) Males Plasma	Males RBC	Males Brain	Females Plasma	Females RBC	Females Brain	Reference
Dogs									
		12 mo							Ahmed and
	0.03 mg/kg/day		88	79*	97	97	71*	108	Sagartz, 1981
	0.1 mg/kg/day	86	68*	107	110	71*	92		
	0.3 mg/kg/day	66*	81*	187	99	78*	78		
n=4		1 mo							Hatch, 1998[c]
	0.3 mg/kg/day		100	92	-	81*	89	-	
	1 mg/kg/day		92	61*	-	81**	93	-	
	3 mg/kg/day		54**	33**	-	44**	37	-	
	3.5 mg/kg/day		46**	28**	-	31**	37	-	
	4.0 mg/kg/day		54**	28**	-	31**	37**	-	
		6 mo							
	0.3 mg/kg/day		92	94	-	69**	79	-	
	1 mg/kg/day		85	63**	-	69**	55	-	
	3 mg/kg/day		-	-	-	-	-	-	
	3.5 mg/kg/day		-	-	-	31**	28**	-	
	4.0 mg/kg/day		46**	28**	-	-	-	-	
		12 mo							
	0.3 mg/kg/day		92	79*	67-100	76	84	100-129	
	1 mg/kg/day		85	52**	91-100	65**	60**	75-91	
	3 mg/kg/day		-	-	-	-	-	-	
	3.5 mg/kg/day		-	-	-	35**	36**	25-91	
	4.0 mg/kg/day		46**	24**	20**-40	-	-	-	

Levels of statistical significance as compared to the controls: * for p#0.05; ** for p#0.01. The reports by Ahmed and Sagartz (1981) and Eiben (1991) provided statistical significance only for p#0.05. Dash ("-") indicated no data.

a/ Studies by Daly and Hogan (1983) and Hatch, 1998 were acceptable for filing the data requirement for the specific type of toxicity test.

b/ N=5 for brain ChE measurements

c/ Brain ChE data were from caudate nucleus, hippocampus, and cerebellum.

"interim" sacrifice. The body weight of the males and females at 50 ppm was approximately 6-8% lower than the controls. Male rats at 50 ppm had an approximately 4-13% increase in food consumption prior to week 13. Food consumption in the 50 ppm females was initially lower (week 1-2), but became higher (approximately 15-20%) throughout the remainder of the study. Based on the food consumption data, the corresponding intake of methyl parathion at week 52 was 0, 0.02, 0.19, and 2.0 mg/kg/day for the males and 0, 0.03, 0.28, and 3.2 mg/kg/day for the females. In addition to the effects on body weight and food intake, a number of effects were also noted at 50 ppm. These included: tremors, alopecia, abnormal gait (in females), peripheral (hind limb) neuropathy with demyelination of the sciatic nerves, retinal degeneration and posterior subcapsular cataracts (in females), increases in brain weight (females, 5%) and heart weight (females, 22%), and a decrease in hemoglobin, hematocrit, and erythrocyte counts. In addition, plasma and brain ChE activities were severely inhibited (brain ChE was reduced to 21-24% of the controls; see Table 5). At 5 ppm, abnormal gait was observed in one female rat and hematological alterations (i.e., decreased hemoglobin, hematocrit, and erythrocyte counts) were noted in the males at the end of the experimental period. At least 50% of the animals had chronic interstitial pneumonia. Mortality was high in all groups including the controls, especially on month 17. Approximately 60-68% of the animals survived after 18th months, and only 33-42% survived to the end of 24 months. This deficiency in animal husbandry compromised a comprehensive evaluation of the chronic toxicity of methyl parathion. Based on plasma and brain ChE inhibition and the many clinical signs of effects, the NOEL was 5.0 ppm (0.19 mg/kg/day). It should be noted that at 5.0 ppm, there was a statistically significant decrease in RBC ChE by 9% at week 26 in the males. In addition, the RBC ChE was decreased by 4-11% in male and female rats at the end of the study, although they were not statistically significant. The NOEL was also 0.5 ppm (0.02 mg/kg/day) based on the neurological signs and hematological effects identified at 5 ppm.

From the above study, USEPA (1997c) established a NOEL of 0.5 ppm (0.02 mg/kg/day) based on RBC ChE inhibition, neurological signs and nerve degenerations, and hematological effects. This formed the basis for the Reference Dose (RfD) determination (USEPA, 1998c, 1999).

The potential ocular and neurological effects of methyl parathion were the focus of an investigation by Daly (1991). This is not a FIFRA guideline study. Groups of 70 Sprague-Dawley rats per sex were fed diets containing 0, 0.5, 2.5, 12.5, or 50 ppm methyl parathion (94.6% pure) for 12 months. The respective doses were 0.02, 0.1, 0.48, and 2.0 mg/kg/day, calculated from the data on body weight and food consumption. Alopecia, cage sores/scabs on paws, yellow stains in the ano-genital area, red nasal discharge, altered gait, and decreased body weight gain (the final body weight was 91% of the controls) were noted at 50 ppm. Plasma, RBC, and brain ChE inhibitions (Table 5) and an increase in peripheral neuropathy occurred at 12.5 and 50 ppm. Neurohistopathological findings are summarized in Table 6. Increased in proximal sciatic and tibial/peroneal nerve myelin degeneration were noted at and above 2.5 ppm. Ophthalmologic, electroretinographic, and neuropathologic investigations did not reveal any ocular effects. The NOEL for brain ChE inhibition was 2.5 ppm (0.1 mg/kg/day). The NOEL for neurological effects was 0.5 ppm (0.02 mg/kg/day). Alternatively, USEPA determined that

the NOEL was 12.5 ppm (0.1 mg/kg/day) for all endpoints, including ChE inhibition (plasma, RBC, brain) and neuropathology (USEPA, 1999).

In October, 1998, DPR received a re-evaluation by Brennecke (1996) the selected peripheral nerve tissues from the aforementioned study by Daly (1991). With an increased number of control and high dose rats examined, the total incidence as presented in Table 6 showed no neuropathological effects at any dose levels. However, the re-evaluation did not follow the procedure set forth by the National Toxicology Program (NTP) for quality assurance in a peer review of pathological findings and convening of a Pathology Working Group for resolving different conclusions between pathologists (USEPA, 1994). Moreover, the background knowledge of dosing regimen for the specimens was not kept from the pathologist making the re-evaluation. DPR therefore determined that the re-evaluation results cannot be used to replace the initial conclusion regarding the neurotoxicity findings.

Table 6. Neurohistopathological findings in Sprague-Dawley CD rats after 12 month of methyl parathion treatment in the diet.

	Concentrations in the diet (ppm[a])									
Myelin	Males					Females				
bubbles[b]	0	0.5	2.5	12.5	50.0	0	0.5	2.5	12.5	50.0
As reported in Daly, 1991										
Proximal Sciatic	0/5	0/5	1/5	2/5	3/5	0/5	1/5	1/5	3/5	3/5
Tibial/Peroneal	0/5	0/5	1/5	2/5	4/5	0/5	0/5	2/5	0/5	3/5
Re-evaluation by Brennecke, 1996										
Total incidence (%)	12/17 (71%)	3/5 (60%)	3/5 (60%)	4/5 (80%)	8/18 (44%)	9/19 (47%)	2/5 (40%)	4/5 (80%)	1/5 (20%)	6/16 (38%)

a/ The respective doses were 0.02, 0.1, 0.48, and 2.0 mg/kg/day, calculated from the data on body weight and food consumption.

b/ Myelin bubbles represented an early myelin degeneration with focal accumulation of fluid within the myelin sheath. In some cases, myelin bubbles were accompanied by myelin phagocytosis and/or Schwann cell proliferation. These changes were similar to the myelin ovoids in the teased nerve.

Nagymajtenyi *et al.* (1995) studied the long-term neurological effects of methyl parathion in Wistar rats using a 3-generation exposure protocol typical of a multi-generation reproductive toxicity study. Methyl parathion at 0.22, 0.33, 0.44, and 0.88 mg/kg/day was administered five times a week through gavage. The dosing schedule was changed to 7 days a week during gestation and lactation periods. Statistically significant changes in EEG (electroencephalogram) index (a quotient of the two fast and the two slow frequency bands) were reported in all three cortical areas (somatosensory, visual, auditory) at as low as 0.22 mg/kg/day. A statistically significant decrease in both the mean amplitudes and an increase in the EEG frequencies were also detected at 0.88 mg/kg/day. The authors stated that the EEG parameters appeared to be more sensitive than ChE inhibition in detecting the neurophysiological effects of OPs, including methyl parathion. Moreover, the effects in the second and third generations were reportedly more pronounced. Unfortunately, the report did not provide sufficient detail for a thorough review.

8.2. Oral Studies in Mice

A chronic study by Eiben (1991) was acceptable under FIFRA guidelines for filling the SB950 data requirements for oncogenicity studies in mice. Groups of 50 B6C3F1/Cr1BR mice per sex were fed diet containing 0, 1, 7, or 50 ppm methyl parathion (95.5% pure) for 104 weeks. Additional groups of 15 mice per sex were included in each dose group for interim sacrifice at 52 weeks. The respective doses were 0, 0.2, 1.6, and 9.2 mg/kg/day for the males and 0, 0.3, 2.1, and 13.7 mg/kg/day for the females. A dose-related brain ChE inhibition was reported in the males at all dose levels (Table 5). At the end of the study, the respective brain ChE activities in male rats from the low to the high dose groups were 81, 77, and 33% of the controls. Substantial brain ChE inhibition was also reported for the females at 50 ppm (38% of the controls). In addition, the activities of RBC ChE were lowered at 7 and 50 ppm in males and females. The respective mortalities for the controls and low to high dose groups were 0, 0, 2, and 6% at week 52 and 16, 0, 20, and 20% at the end of the study. Contrary to the body weight reductions noted in the subchronic studies (Daly and Rinehart, 1979, 1980b), mice at the high dose showed a substantial body weight increase over the controls. By the end of the study, the average body weight of mice at 50 ppm was approximately 10-20% higher than the controls while the food consumption was 13-15% lower. An increase in the absolute weight of liver, kidneys, and brain and a decrease in their relative weights were also noted at 50 ppm, more prominently in the males. There was a dose-related elevation of plasma cholesterol levels that was statistically significant ($p<0.01$) in females at 7 and 50 ppm and males at 50 ppm. In the males, the number of animals noted as having “poor general condition” increased to 8 to 9 animals per dose group at 7 and 50 ppm compared to one animal in the controls. As the dose increased, the lag time for the condition appeared to be shortened (starting on day 2 at 50 ppm compared to day 99 in the controls). Tremors were noted in one male during week 2. Paralysis was noted in one female during week 84. Gross and histopathological examinations did not show any treatment-related effects. Based on the dose-related inhibition of brain ChE, the lowest tested dose of 1 ppm (0.2 mg/kg/day) could be the LOEL, although it is a NOEL based on the statistical analysis since the ChE inhibition was only statistically different

($p<0.05$) from the control at the mid and high doses. However, the statistical power was compromised by the low sample size (5 per dose group). Based on the body and organ weight changes and poor general conditions in the males, the NOEL was 7 ppm (1.6 mg/kg/day).

8.3. Oral Studies in Dogs

Two one-year studies are on file in DPR. In the study by Ahmed and Sagartz (1981), groups of 8 dogs per sex received 0, 0.03, 0.1 or 0.3 mg/kg/day methyl parathion (93.65% pure) through the diet for one year. A reduction in brain ChE activity (down to 78% of the controls) was noted in females at 0.3 mg/kg/day (Table 5). No clinical signs were noted. Males at 0.1 and 0.3 mg/kg/day had an occasional decrease in feed consumption during week 15 and week 30 but this did not result in significant changes in the overall body weight gain. The study was deficient in that the dose selection was not justified and the potential for ophthalmologic effects was not adequately tested. Based on the brain ChE inhibition, the NOEL was 0.1 mg/kg/day. Although the dose level used in this study had apparently not reached the maximum tolerated dose (MTD), the requirement for conducting a study with sufficiently high dose was judged by DPR as no longer necessary because a lower NOEL identified in the rat studies was suitable for risk assessment purposes.

In a recent study, Hatch (1998) investigated the ocular toxicity of methyl parathion. Groups of 4 dogs per sex received 0, 0.3, 1.0, 3.5, and 4.0 mg/kg/day methyl parathion (95.8% pure) through the diet for one year. At 3 months of exposure, the dose of two males was changed from 3.5 mg/kg/day to 4 mg/kg/day while the dose for two females was changed from 4.0 mg/kg/day to 3.5 mg/kg/day. The remaining two dogs from each group were killed. Ocular toxicities were also studied in groups of 4 dogs per sex that received 3.0 mg/kg/day methyl parathion for 3 months and after a 30-day subsequent recovery period (i.e., given untreated diet). No treatment-related ophthalmological changes (intraocular pressure, electroretinogram) were reported at 3, 6, and 12 months of exposure at any dose levels. At 3 mg/kg/day, occasional tremors were noted in two females during week 4 to 10 while all four females had diarrhea. Effects reported at 3.5 and 4.0 mg/kg/day included diarrhea, thinness, statistically significant increase in relative adrenal weight (adrenal to brain ratio), decreased absolute and relative spleen weight, and lymphoid cell depletion in thymus. Plasma, RBC, and brain ChE activities showed dose-related decreases (Table 5). Plasma ChE inhibition occurred in the females at all dose groups, and the effects at 0.3 mg/kg/day were statistically significant (p#0.01) at the 1 and 6 months measurements. At this dose, the RBC ChE inhibition in the males was also statistically significant at the end of the study. Substantial brain ChE inhibition was also noted at 3.5 and 4 mg/kg/day. As much as 25% brain inhibition was noted at 1 mg/kg/day, although not reported as statistically significant. Based on the statistical significance in plasma and RBC ChE inhibition, the lowest dose of 0.3 mg/kg/day was the LOEL. The NOEL for a 25% brain ChE inhibition was 0.3 mg/kg/day. The NOEL for the clinical signs of toxicity was 1.0 mg/kg/day.

9. ONCOGENICITY

Three studies in rats and 2 studies in mice were available for the evaluation of the human oncogenic potential of methyl parathion. Detailed descriptions for two chronic studies in rats by Bomhard *et al*. (1981) and Daly and Hogan (1983) and one study in mice by Eiben (1991) were presented in Section 8., *Chronic Toxicity*. Only data pertaining to the oncogenic effects from these studies are given in this section. In addition, the NCI study (NCI, 1979) in rats and mice is presented.

9.1. Oral Studies in Rats

Limited evidence of oncogenicity was demonstrated in the following three studies. Somewhat elevated incidences of adrenal adenomas in the males and uterus adenocarcinomas in the females were noted in two of the three studies. A marginally statistically significant increase of thyroid adenomas was also noted in one study. The overall oncogenicity evidence in rats is generally "equivocal" or "limited" at best. The current data are insufficient for speculating on the possible implication of these results to human health at the exposure levels commonly experienced by humans.

Study by Bomhard *et al.* (1981)

In the 2-year study with Wistar rats by Bomhard *et al*. (1981), various types of tumors were found in a total of 15 organ/tissue sites. The authors reported that all were within the range of spontaneous rates and were primarily the result of aging. Table 7 presents the incidences of tumor sites that had at least a 10% occurrence in any dose group. There were apparent slight increases in thyroid adenomas in the males and uterus adenocarcinomas in the females. The increase in thyroid adenomas was marginally significant statistically ($p<0.05$) by the Fisher exact pair-wise comparison.

Bomhard and Rinke (1994) published a set of spontaneous tumor incidences in Wistar rats. The historical data were a collection of tumor incidences from the controls (approximately 50 rats per sex per study) of 22 two-year studies conducted in the Institute of Toxicology of BAYER AG in Germany between May 1975 and December 1980. This historical database is a pertinent reference for the methyl parathion study because it was from the same laboratory, by the same author, and included the study period of the methyl parathion study. The concurrent control incidences of adrenal tumors in males and females (Table 7) were on the high end of the historical range and substantially higher than the average spontaneous rates. An exact comparison of the incidence of thyroid tumors cannot be made with the historical data because the report by Bomhard *et al*. (1981) did not specify whether they were follicular or c-cell tumors. The concurrent control incidence in the males and females were within the historical range of c-cell benign or malignant tumors but exceeded the range for follicular adenomas or carcinomas.

Table 7. Tumor incidences from a 2-year feeding study in Wistar rats conducted by Bomhard *et al.* (1981)[a]

Sex/Tumor site/type	0 ppm	2 ppm	10 ppm	50 ppm
(M) Adrenal medulla				
pheochromocytoma	14/49 (28.6%)	3/50 (6.0%)	4/49 (8.2%)	3/47 (6.4%)
(F) Adrenal medulla				
pheochromocytoma	3/49 (6.1%)	0/48 (0%)	0/50 (0%)	0/41 (0%)
(M) Pituitary				
adenoma	10/49 (20.4%)	13/50 (26.0%)	11/49 (22.4%)	4/47 (8.5%)
adenocarcinoma	1/49 (2.0%)	0/50 (0%)	0/49 (0%)	0/47 (0%)
combined	11/49 (22.4%)	13/50 (26.0%)	11/49 (22.4%)	4/47 (8.5%)
(F) Pituitary				
adenoma	6/49 (12.2%)	7/48 (14.6%)	16/50 (32.0%)	3/41 (7.3%)
adenocarcinoma	0/49 (0%)	0/48 (0%)	0/50 (0%)	1/41 (2.4%)
combined	6/49 (12.2%)	7/47 (14.6%)	16/50 (32.0%)	4/41 (9.8%)
(M) Thyroid				
adenoma[b]	4/49 (8.2%)	3/50 (6.0%)	2/49 (4.1%)	12/47 (25.5%)*
adenocarcinoma	1/49 (2.0%)	0/50 (0%)	0/49 (0%)	0/47 (0%)
combined	5/49 (10.4%)	3/50 (6.0%)	2/49 (4.1%)	12/47 (25.5%)*
(F) Thyroid				
adenoma	3/49 (7.3%)	5/48 (10.4%)	9/50 (18.0%)	3/41 (7.3%)
adenocarcinoma	0/49 (0%)	0/48 (0%)	0/50 (0%)	1/41 (2.4%)
combined	3/49 (7.3%)	5/48 (10.4%)	9/50 (18.0%)	4/41 (9.8%)
(F) Uterus				
adenoma	0/49 (0%)	1/48 (2.1%)	0/50 (0%)	0/41 (0%)
adenocarcinoma[c]	4/49 (8.2%)	3/48 (6.3%)	9/50 (18.0%)	8/41 (19.5%)
combined	4/49 (8.2%)	4/48 (8.3%)	9/50 (18.0%)	8/41 (19.5%)
(F) Mammary gland				
adenoma	1/49 (2.0%)	0/50 (0%)	0/50 (0%)	1/41 (2.4%)
adenocarcinoma	0/49 (0%)	0/50 (0%)	1/50 (2.0%)	0/41 (0%)
combined	1/49 (2.0%)	0/50 (0%)	1/50 (2.0%)	1/41 (2.4%)

a/ The incidence was based on animals at risk (animals survived past week 52). Only sites that were highlighted in all 3 rat oncogenicity studies were listed.

b/ The incidence at 50 ppm exceeded the highest possible range of the historical incidence (highest: 23.5%, average: 6.5%) based on 1211 control animals.

c/ The incidences at the 10 and 50 ppm levels exceeded the highest range of historical incidence (0-16.3%, average: 7.8%) based on 1236 control animals.

* Fisher exact test at $p<0.05$.

Nevertheless, it is important to note that, the incidences of the thyroid adenomas, although unspecified regarding the cell type, would have exceeded the historical incidence. This is illustrated by the following analysis. The historical incidence was 0-4.4% (ave. 1.1%) for follicular cell adenomas and 0-19.1% (ave. 5.4%) for benign c-cell tumors (Bomhard and Rinke, 1994). Thus, the highest possible historical incidences for both types of benign thyroid tumors would be 23.5%. Accordingly, the 25.5% incidence of thyroid adenomas in males at 50 ppm remained outside of this highest possible historical incidence.

A comparison to the historical control incidence was also made regarding of the uterus adenocarcinomas. Although the incidence at the mid and high dose levels were not statistically significantly different from the concurrent controls, they exceeded the historical range while the incidence of controls (8.2%) stayed well within the range. The respective incidences at the mid (10 ppm) and high (50 ppm) dose levels were 18.0% and 19.5% while the historical controls ranged from 0 to 16.3% with an average of 7.8%.

Study by Daly and Hogan (1983)

The lifetime study by Daly and Hogan (1983) in Sprague-Dawley CD rats was accepted for filling the SB950 data requirement of oncogenicity testing in rats. Nevertheless, the evaluation of the oncogenic potential was compromised due to the excessive early mortality (only 33-42% survival at the end of 24 months) and the high incidence (greater than 50%) of chronic interstitial pneumonia in all dose groups. It should also be noted that, in spite of the generally high mortality, the surviving animals were kept beyond the common study period of 2 years. Except for the 5 animals per group killed on day 736 ("interim" sacrifices), the study was not terminated until month 25 for the males and month 28 for the females. The study report highlighted the occurrences of pituitary adenoma, adrenal adenoma and carcinoma, and mammary gland tumors in female rats. However, it stated that they were not treatment related because the incidences were not different between the controls and the treatment groups.

Table 8 summarizes the incidences of endocrine and other tumors highlighted in both this study and the aforementioned study by Bomhard *et al.* (1981) in Wistar rats. Excluded from the table were pituitary tumors in male and female rats and adrenal tumors in the females because the control incidences were very high: 37.5% for male pituitary adenomas, 71.2% for female pituitary adenomas, and 53.3% for female adrenal tumors. Almost all tumors in the thyroid, mammary gland, and uterus were detected on or beyond day 736, except the thyroid carcinoma detected on day 523 in a female rat at 0.5 ppm and the uterus stromal sarcoma detected on day 401 in a control rat. None of the tumor incidences in the treatment groups were statistically significantly different from the concurrent controls.

Three sets of historical incidences are available for comparison: The data on Sprague-Dawley CD® rats (Crl:CD®BR) compiled by McMartin *et al.* (1992) from 9 studies between 1984-91,

Table 8. Tumor incidences from a lifetime feeding study in Sprague-Dawley CD rats conducted by Daly and Hogan (1983)[a]

Sex/Tumor site/type	0 ppm	0.5 ppm	5.0 ppm	50 ppm
(M) Adrenal cortex[b]				
adenoma	9/57 (15.8%)	9/56 (16.1%)	12/57 (21.1%)	12/59 (20.3%)
carcinoma	0/57 (0%)	1/56 (1.8%)	0/57 (0%)	1/59 (1.7%)
combined	9/57 (15.8%)	10/56 (17.9%)	12/57 (21.1%)	13/59 (22.0%)
(M) Thyroid follicular cell				
adenoma	0/56 (0%)	0/55 (0%)	0/57 (0%)	0/54 (0%)
carcinoma	0/56 (0%)	0/55 (0%)	1/57 (1.8%)	2/54 (3.7%)
combined	0/56 (0%)	0/55 (0%)	1/57 (1.8%)	2/54 (3.7%)
(F) Thyroid follicular cell				
adenoma	1/57 (1.8%)	0/60 (0%)	0/58 (0%)	2/53 (3.8%)
carcinoma	0/57 (0%)	3/60 (5%)	1/58 (1.7%)	1/53 (1.9%)
combined	1/57 (1.8%)	3/60 (5%)	1/58 (1.7%)	3/53 (5.7%)
(F) Mammary gland				
adenoma	1/58 (1.7%)	0/54 (0%)	0/56 (0%)	4/52 (7.7%)
carcinoma	5/58 (8.6%)	1/54 (1.9%)	3/56 (5.4%)	3/52 (5.8%)
combined	6/58 (10.3%)	1/54 (1.9%)	3/56 (5.4%)	7/52 (13.5%)
(F) Uterus endometrial				
adenocarcinoma[c]	0/60 (0%)	1/60 (1.7%)	0/60 (0%)	1/54 (1.9%)
stromal sarcoma	1/60 (1.7%)	0/60 (0%)	1/60 (1.7%)	1/54 (1.9%)
Undifferentiated neoplasm	0/60 (0%)	0/60 (0%)	0/60 (0%)	1/54 (1.9%)

a/ The study continued for 25 months in the males and 27.6 months in the females. The incidences were based on animals at risk (animals survived beyond 52 weeks or the first appearance of tumors) and excluded animals whose specific tissues were not examined.

b/ The incidence at 5.0 and 50 ppm exceeded the range of historical incidence (0-3%, ave. 1.6% and 1.4-16.4%, ave. 2.88%) in the two available databases in the literature.

c/ The incidence at 5.0 and 50 ppm exceeded the range of historical incidence (0-1.4%) in the two available databases in the literature.

the data compiled by Chandra *et al*. (1992) from 17 studies between 1986-92, and the data compiled by Lang (1992) from up to 36 studies with VAF® rats between April 1984 to February 1989. The data reported by Chandra *et al*. (1992) consisted only of the average incidence without the ranges. It appears that substantial variation in the historical data exists between conducting laboratories. The concurrent controls from the study by Daly and Hogan (1983) generally stayed within these wide historical ranges, albeit the incidences at some sites were at the high end of the historical range.

The incidence of adrenal adenomas in the males at 5.0 ppm (21.1%) and 50 ppm (20.3%) exceeded the historical range. The reported historical range was 0-3% (ave. 1.6%) by McMartin *et al*. (1992) and 1.4-16.4% (ave. 2.88%) by Lang (1992). It should be noted that adrenal cortex adenomas were also found in F_0 male rats in a 2-generation reproduction study by Daly and Hogan (1982) (see: Section 11., *Reproductive Toxicity*). Among the 10 male rats per treatment groups (0, 0.5, 5.0, 25 ppm methyl parathion in the diet) that were examined histopathologically after only 23 weeks of exposure, 2 rats at the high dose had adenomas in the adrenal cortex while no tumors were found in any other groups. This incidence rate of 20% also exceeded the historical range provided in McMartin *et al*. (1992) and Lang (1992). In a rebuttal to the evaluation by DPR (formerly under Department of Food and Agriculture) regarding the reproductive toxicity study, the registrant (A/S Cheminova, 1990) noted a historical range of 1.6 to 33% for adrenal cortex adenomas from the conducting laboratory Bio/dynamics. The high end of the range was much higher than the 16.4% recorded in the open literature. The claim of a historical range as high as 33% cannot be validated because the database for the quoted range was not provided in the rebuttal.

One animal per dose group at the low (0.5 ppm) and high (50 ppm) dose groups had uterus endometrial adenocarcinoma while none was reported in the concurrent controls. These tumors appeared to be rare. The reported historical range compiled by McMartin *et al*. (1992) was 0-1.4% (ave. 0.5%) from a total of 584 animals. The historical data selected from 7 out of 13 sets of data compiled by Lang (1990) to represent the same period of study (1980-85) showed a range of 0-2.8% (ave. 0.7%) out of 421 animals. This included five sets of data with the zero incidence 0/259 (0%) and two sets at 1/90 (1.1%) and 2/72 (2.8%).

Study by NCI (1979)

A third oncogenicity study was conducted by NCI (1979) in Fischer F344 rats. Groups of 50 rats per sex were fed diets containing 20 or 40 ppm methyl parathion (94.6% pure) for 105 weeks. A group of 20 untreated rats per sex served as matched controls. Decreases in the mean body weight at the treatment groups were reported as dose-related. Females at 40 ppm had a much higher mortality rate. The respective survival rates for the control, mid, and high dose groups were 96%, 83% and 46% at the end of the study. None of the tumor incidences in the treatment groups were statistically significantly different (by both Fisher exact test and Cochran-Armitage trend test) from the incidence in the 20 matched controls. The comparison was somewhat limited by the low number of animals in the control groups. Tumor incidences of the specific sites and types highlighted in the previous two studies

are presented in Table 9. The tumor types selected for the tabulation also encompassed those that appeared to have higher incidences at the treatment groups from this study, specifically tumors in the adrenal cortex and thyroid follicular cells. For the same reason as noted for the previous study, the incidence of pituitary tumors was not included in the table because of an overall high incidence in all groups (45% and 60% in the males and female controls). The study did not report the incidences of adenoma or carcinoma in the mammary gland or uterus. No evidence of oncogenicity was indicated in this study.

Table 9. Tumor incidences from a 105-week feeding study in F344 rats conducted by the National Cancer Institute (NCI, 1979)[a]

Tumor site/type	0 ppm	20 ppm	40 ppm
Adrenal cortex adenoma			
Males	0/20 (0%)	0/50 (0%)	2/50 (4.0%)
Females	1/20 (5.0%)	2/50 (4.0%)	0/47 (0%)
Adrenal pheochromocytoma			
Males	3/20 (15.0%)	6/50 (12.0%)	5/50 (10.0%)
Females	0/20 (0%)	1/50 (2.0%)	0/47 (0%)
Thyroid follicular cell carcinoma			
Males	0/20 (0%)	0/48 (0%)	1/49 (2.0%)
Females[b]	-	-	-
Thyroid C-cell adenoma/carcinoma			
Males	3/20 (15.0%)	3/48 (6.3%)	3/49 (6.1%)
Females	1/20 (5.0%)	1/49 (2.0%)	0/47 (0%)
Mammary gland fibroadenoma			
Males	0/20 (0%)	1/50 (2.0%)	0/50 (0%)
Females	5/20 (25%)	2/50 (4.0%)	0/47 (0%)

a/ The incidences were based on the number of animals examined. No incidence of mammary gland adenoma or carcinoma was reported.

b/ Not reported.

9.2. Oral Studies in Mice

Under the experimental conditions, no statistically significant increase in tumor incidences was found in the two available studies in B6C3F1 mice.

Study by Eiben, 1991

The study by Eiben (1991) in B6C3F1 mice was judged acceptable for filling the SB950 data requirement of testing in mice. The report concluded that no increase in the tumor incidence was evident in this study. The only tumor that showed some increase in the treatment groups was the lung bronchiolo-alveolar tumor. The incidences in the male and females mice are presented in Table 10. The incidences in the treatment groups were not statistically different from the controls. The toxicities at the high dose groups were previously described (see: Section 8.2). Plasma, RBC, and brain ChE measured at 52 and 104 weeks were only 11-54% of the controls (Table 5). Other significant effects included increased death (6%) at week 52, reduced (13-15%) food consumption accompanied by increased body weight (10-20%), decreased relative organ weights, increased incidence of animals with "poor general condition", and the appearance of cholinergic signs (i.e., tremors, paralysis). Based on these effects, it was judged that the dose range was sufficiently high to meet the FIFRA study requirement.

Table 10. Tumor incidences from a 2-year study in B6C3F1 mice conducted by Eiben (1991)[a]

Sex/Tumor site/type	0 ppm	1 ppm	7 ppm	50 ppm
(M) Lung, bronchiolo-alveolar				
adenoma	1/50 (2.0%)	6/50 (12.0%)	4/49 (8.2%)	4/46 (8.7%)
carcinoma	2/50 (4.0%)	3/50 (6.0%)	3/49 (6.1%)	1/46 (2.2%)
combined	3/50 (6.0%)	9/50 (18.0%)	7/49 (14.3%)	5/46 (10.9%)
(F) Lung, bronchiolo-alveolar				
adenoma	2/49 (4.1%)	1/48 (2.1%)	1/49 (2.0%)	2/47 (4.3%)
carcinoma	0/49 (0%)	0/48 (0%)	0/49 (0%)	1/47 (2.1%)
combined	2/49 (4.1%)	1/48 (2.1%)	1/49 (2.0%)	3/47 (6.4%)

a/ The incidence was based on animals at risk (animals survived past week 52).

Study by NCI, 1979

No evidence of oncogenicity was found in the bioassay conducted by the NCI in B6C3F1 mice (NCI, 1979). Groups of 50 mice per sex were initially fed diets containing 62.5 or 125 ppm methyl parathion (94.6% pure) for 37 weeks. The dose levels for the respective groups of males were then reduced to 20 and 50 ppm for the remaining 65 weeks of the bioassay because of a substantial decrease in body weight gain (down to approximately 70-86% of the controls) by week 37. The time-weighted-average levels in the diet during the entire experimental period were 35 and 77 ppm. The control groups consisted of only 20 untreated rats per sex. The body weight of mice at the high dose level was slightly lower than the controls. No effects on mortality or tumor occurrences were noted at any treatment levels. Similar to the aforementioned study by Eiben (1991), the lung was the only site that showed an apparent increase in the incidence, although they were not statistically different from the controls. The incidence data are summarized in Table 11.

Table 11. Tumor incidences from a 104-week feeding study in B6C3F1 mice conducted by the National Cancer Institute (NCI, 1979)[a]

Tumor site/type	0 ppm	62.5 ppm[b]	125 ppm[c]
(M) Lung Alveolar/Bronchiolar			
adenoma	1/19 (5.3%)	5/50 (10.0%)	5/49 (10.2%)
Carcinoma	0/19 (0%)	5/50 (10.0%)	3/49 (6.1%)
combined	1/19 (5.3%)	10/50 (20.0%)	8/49 (16.3%)
(F) Lung Alveolar/Bronchiolar			
adenoma	0/19 (0%)	1/49 (2.0%)	1/48 (2.1%)
Carcinoma	0/19 (0%)	2/49 (4.1%)	1/48 (2.1%)
combined	0/19 (0%)	3/49 (6.1%)	2/48 (4.2%)

a/ The incidences were based on the number of animals examined.

b/ The dose in the males was reduced to 20 ppm after week 37. The time-weighted-average dose was 35 ppm.

c/ The dose in the males was reduced to 50 ppm after week 37. The time-weighted-average dose was 77 ppm.

10. GENOTOXICITY

Methyl parathion has been tested for genotoxic potential in many *in vitro* and *in vivo* systems. Reports of studies by Herbold (1980, 1982a,b, 1984) and Curren (1989) are on file at DPR. These studies were judged sufficient for filling the SB950 data requirement of genotoxicity testing. Methyl parathion was tested positive by the Ames tests. In addition, many genotoxicity studies on methyl parathion were also available in the published literature. Collectively, they showed that methyl parathion is genotoxic in laboratory studies. The overall weight of evidence indicates that methyl parathion has the potential to cause changes in genetic materials in humans.

The results of *in vitro* and *in vivo* gene mutation assays are summarized in Table 12. Methyl parathion induced base-pair substitution in *S. typhimurium* strains TA100 and TA1535, but not in *E. coli*. Methyl parathion was positive in one of the two sex-linked recessive lethal assays with *Drosophila melanogaster*.

The results of assays on chromosomal damage are summarized in Table 13. Except for the study by Kumar *et al.* (1993), all are *in vivo* studies. The micronucleus tests in rats and mice were positive except in the mouse study by Herbold (1982b). The dose level in this study may not be sufficiently high, since no toxicity was observed. Two of the three chromosomal aberration tests in Wistar rats showed positive results. The negative study by Nehez *et al.* (1994) was a longer term study at a lower dose range. The two chromosomal aberration tests in mice were negative. However, chromosomal aberrations were detected among humans after acute suicidal or occupational exposures (van Bao *et al.*, 1974) and in cotton field workers exposed to a number of pesticides including methyl parathion (Rupa *et al.*, 1989). The four dominant lethal tests in mice showed negative results.

Test results on DNA binding, damage, and repair capabilities are summarized in Table 14, with results on sister chromatid exchange presented in Table 15. *In vivo* and *in vitro* binding of methyl parathion to DNA was reported in rats and mice (Bartoli *et al.*, 1991). The *in vitro* binding appeared to require the activation of methyl parathion by P450-dependent mixed function oxidases and microsomal GSH-transferases. DNA damage was also demonstrated in repair-deficient *S. typhimurium* (TA1538/TA1978) (Rashid and Mumma, 1984). A 10-fold elevation of DNA breakage in Col-E1 plasmid was reported (Griffin and Hill, 1978). Marginal induction of mitotic recombination was also reported in *Saccharomyces cerevisiae* D3 (Simmon *et al.*, 1977). No unscheduled DNA synthesis (UDS) was detected in either human WI-38 cells (Simmon *et al.*, 1977) or rat primary hepatocytes (Curren, 1989). The UDS assay detects DNA damage through monitoring the DNA repair capacity. It is relatively insensitive as a mutagenicity screen; i.e., negative results do not refute the potential for mutagenicity while positive results are highly indicative of the mutagenicity potential of a chemical. Methyl parathion was not shown to cause lethality in repair-deficient strains of *E. coli* and *B. subtilis* (Simmon *et al.*, 1977; Rashid and Mumma, 1984).

Table 12. Effects of methyl parathion on gene mutation[a]

Test System/Strain[b]	Route/ Dose	Activation	Results	References	Comments
S. typhimurium TA100, TA1535, TA1537, TA1538	1, 5, 10, 50, 100, 500, 1000 Fg/plate (80% pure)	±; m	neg	Simmon *et al.*, 1977	# plates/dose not specified. Positive control for only TA1538 (+S9). No statistical difference between controls and treatment.
S. typhimurium TA98, TA100, TA1535, TA1538	20, 100, 500, 1000, 2000, 2500, 4000, 8000 Fg/plate (94.3-94.5% pure)	±; r	pos	Herbold, 1980	4 plates/dose. No control for -S9 series. Positive in TA100 (+S9) and TA1535 (+S9).
S. typhimurium TA98, TA100, TA1535, TA1537	20, 100, 400, 500, 600, 625, 780, 900, 1000-10000 Fg/plate (95.6-96.1% pure)	±; r	pos	*Herbold, 1982a	4 plates/dose. Equivocal results in TA98(+S9). Positive in TA100 (+S9).
S. typhimurium TA98, TA100, TA1535, TA1537	10, 33, 100, 333, 445 667, 1000, 1500, 2000 Fg/plate (purity ns)	±; r, h	pos	Haworth *et al.*, 1983	Preincubation procedure used. # plates/dose not specified. Positive results in TA100 (+S9).
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	5, 10, 25, 50, 100, 125, 250, 325, 635 1250 Fg/plate (99.4% pure)	±; r	pos	Rashid and Mumma, 1984	3-5 plates/dose. Dose-related increase in TA100 (+ S9) (>2-fold at high dose). Cytotoxic at two highest doses in TA1537 (+S9).
S. typhimurium TA100	0.5, 5, 50, 500 Fg/plate (purity ns)	±; ns	pos	Breau *et al.*, 1985	3 plates/dose. Positive at 500 Fg/ml.

(continued)

Table 12. Effects of methyl parathion on gene mutation[a] (cont.)

Test System/Strain[b]	Route/ Dose	Activation	Results	References	Comments
S. typhimurium TA100NR	50, 100 Fg/plate (purity ns)	-	neg	Vijayaraghavan and Nagarajan, 1994	no details given
E. coli WP2 WP2uvrA WP67 CM611, CM571	250, 500, 1000, 2000 Fg/plate (99.4% pure)	±; r	neg	Rashid and Mumma, 1984	3-5 plates/dose. Effect significant at the highest dose (+S9) with WP2,WP2uvrA, and WP67, < 2-x of the control frequency.
E. coli WP2	1, 10, 50, 100 Fg/plate (80% pure)	±; m	neg	Simmon *et al.*, 1977	# plates/dose not specified. No treatment-related effects (ave # revertants/plate).
SLRL *D. melanogaster*	0.25 and 5.0 ppm (purity ns)	feed	neg	Waters *et al.*, 1980	Sample size not specified.
SLRL *D. melanogaster* M-5	0.0315, 0.063, 0.125 ppm (purity NS) to 24, 48, & 72 hrs larvae for 96, 72 & 48 hrs, respectively.	feed	pos	Tripathy *et al.*, 1987	66-78 males/exposure time/dose. 3 of the 75 24-hr larvae at 0.063 ppm had 2 lethal chromosomes each, indicative of mutation in mitotically dividing spermatogonia.

* Studies acceptable for filing the SB950 data requirements.

a/ Abbreviations: ns, purity not stated; ±, with and without microsomal S9 fraction; h, hamster liver microsomal S9 fraction; m, mouse liver microsomal S9 fraction; r, rat liver microsomal S9 fraction; pos: positive results; neg, negative results.

b/ *Salmonella typhimurium*; *Escherichia coli*, *Drosophila melanogaster*; SLRL = Sex-linked recessive lethal test.

Table 13. Effects of methyl parathion on chromosomal damage - *in vivo* studies[a].

Test	Species/ cell line	Route/ Dose	Activation	Results	References	Comments
Micro-nuclei	Rat (Wistar)	1, 2, 4 mg/kg (purity ns); single dose	i.p.	pos	Grover & Malhi, 1985	5 males/dose; Bone marrow examined 30 hours after dosing. Effect dose-related.
Micro nuclei	Rat (Wistar)	3, 5 mg/kg (purity ns); single dose	i.p.	pos	Vijayaraghavan & Nagarajan, 1994	4 rats/group, bone marrow examined 1 and 2 days after dosing; ~ 4-fold increase at 5 mg/kg on day 2; statistically significant
Micro-nuclei	Mouse (Bor: NMRI)	5, 10 mg/kg; (95.6-96.1% pure); 2 days	gavage	neg	Herbold, 1982b	5 mice/sex/dose. Bone marrow examined 6 hours after second dosing. No toxicity. Treatment not different from controls ($p<0.05$).
Micro-nuclei	Mouse (Swiss)	9.4, 18.8, 37.5, 75 mg/kg, (purity ns); single dose	gavage	pos	Mathew *et al.*, 1990	4 females/time/dose. Bone marrow examined 24, 48, & 72 hours after dosing. Effect dose-related.
Chrom. Aberration	Rat (Wistar)	0.5, 1.0, 2.0 mg/kg; (purity ns); 5 days	i.p.	pos	Mahli & Grover, 1987	5 males/dose, 30-35 cells/rat. Bone marrow examined 24 Hours after last dose. Effect dose-related.
Chrom, Aberration	Rat (Wistar)	3, 5 mg/kg (purity ns); single dose	i.p.	pos	Vijayaradhavan & Nagarajan, 1994	200 bone marrow cells/rat, 2.5-5-fold increase; statistically significant.

(continued)

Table 13. Effects of methyl parathion on chromosomal damage - *in vivo* studies[a] (cont.).

Test	Species/ cell line	Route/ Dose	Activation	Results	References	Comments
Chrom. Aberration	Rats (Wistar)	0.25, 0.33, 0.50 mg/kg; (90% pure); 6 wks	gavage	neg	Nehez *et al.*, 1994	10 male rats per group; chromosomes from bone marrow cells examined 1 day after the last treatment.
Chrom. Aberration	Mouse (Q)	10 mg/kg (99% pure), 1 dose	i.p.	neg	Degraeve *et al.*, 1984a	20 males/dose. Spermatocytes examined 10-15 days after dosing. Treatments not different from controls (Chi-square test)
Chrom. Aberration	Mouse (Q)	0.15 ppm (99% pure), 5 d/wk, 7 wks	drinking water	neg	Degraeve *et al.*, 1984b	8 males/dose. Bone marrow, spermatogonia, and spermatocytes examined after 7 wks. No treatments-related difference (Chi-square test).
Chrom. Aberration	Human	unknown dose of Wofatox; acute	oral/ exposure	pos/neg other	van Bao *et al.*, 1974	57-100 lymphocytes/person, 4 suicides, 1 worker; age 20-53. Significant effect at 1 month. No effect immediately or 6 months after exposure.
Chrom. Aberration	Human	unknown dose; chronic (1-9 months)	unknown	neg	de Cassia Stocco *et al.*, 1982	200 lymphocytes/person, 15 male workers (age 19-49); blood ChE < 75% of controls (13 men; age 19-38). No statistically significant effects.
Chrom. Aberration	Human	unknown dose; chronic (5-25 yrs) exposure to cotton pesticides	oral/others	pos	Rupa *et al.*, 1989	200 lymphocytes/person, 52 male workers (age 21-47). Increased aberrations over the controls (age 22-42; 25 male workers).

(continued)

Table 13. Effects of methyl parathion on chromosomal damage - *in vivo* studies[a] (cont.).

Test	Species/ cell line	Route/ Dose	Activation	Results	References	Comments
Chrom. Aberration	Human	0.02, 0.04, 0.08, 0.16 Fg/ml	*in vitro*	neg	Rupa *et al.*, 1990	tested 3 times; treated for 24, 48, or 72 hrs. reported no effects (data not shown)
Chrom. Aberration	Human	0.08, 0.16 ug/ml (98% pure)	*in vitro*	pos/neg	Kumar *et al.*, 1993	used peripheral lymphocytes; statistically significant at 0.16 ug/ml in chronic smokers (alcoholics or not); no effects in non-smokers.
Dominant Lethal	Mouse (ICR/ SIM)	20, 40, 80 ppm (80% pure); 7 wks	feed	neg	Simmon *et al.*, 1977	20 males/dose. Mated 1:2 weekly for 8 weeks at the end of 7 week exposure. No statistical difference (Chi-square test; p>0.01)
Dominant Lethal	Mouse (Bor: NMRI)	10 mg/kg (95.7% pure); single dose	gavage	neg	*Herbold, 1984	46-50 males/dose. Mated 1:1 every 4 day; 48 days. No statistical difference (p>0.05)
Dominant Lethal	Mouse (Q)	0.15 ppm (99% pure), 5 d/wk, 7 wks	drinking water	neg	Degraeve *et al.*, 1984b	20 males/dose. Mated 1:4; 1 week. Examined 14 days after mating. No statistical difference (Chi-square test).
Dominant Lethal	Mouse (Q)	10 mg/kg (99% pure) single dose	i.p. 1 dose	neg	Degraeve & Moutschen, 1984	5 males/dose. Mated 1:4 weekly; 7 wks. No statistical difference (Chi-square test).

* Study acceptable for filing the SB950 data requirements.

a/ Abbreviations: Chrom, Chromosomal; ns, purity not stated; i.p., intraperitoneal injection.

Table 14. Effects of methyl parathion on DNA[a].

Test	Species/ Cell line	Route/ Dose	Activation	Results	References	Comments
DNA Binding	Rat (Wistar) Mouse	1.31, 0.033 Fmol/kg[b] (99% pure)	i.p.	pos	Bartoli *et al.*, 1991	*In vivo* (1.31 Fmol/kg): 6 male rats, 24 mice; binding to liver, kidney, lung macromolecules (DNA, RNA, proteins). *In vitro* (0.033 Fmol/kg): microsomal/cytosolic fractions of organs of 20 treated rats; binding to calf thymus DNA.
UDS	Rat Primary Hepatocytes	0.0003, 0.001, 0.003, 0.01, 0.02, 0.03 *Fl*/ml (purity ns)	–	neg	*Curren, 1989	150 cells/dose. No dose-related increase; no significant increase of net nuclear count ≥ 5.
UDS	Human WI-38	10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} M (80% pure)	±; m	neg	Simmon *et al.*, 1977	3-6 replicates/dose. No treatment-related effect.
DNA damage	*S. typhimurium*; TA1538/ TA1978	250, 500, 1000 ug/disc (99.4% pure)	–	pos	Rashid & Mumma, 1984	3 plates/dose. Dose-related increase (2-5 fold) in repair-deficient TA1538.
DNA damage	*E. coli* W3110/ P3478	1 mg/disc (80% pure)	–	neg	Simmon *et al.*, 1977	3 replicates. No difference in the diameter of inhibition zone.
DNA damage	*E. coli* K-12; WP2	250, 500, 1000 ug/disc (99.4% pure)	–	neg	Rashid & Mumma, 1984	3 plates/dose. No difference in the diameter of inhibition zone with strains deficient in DNA polymerase A and repair mechanism.

(continued)

Table 14. Effects of methyl parathion on DNA[a] (cont.).

Test	Species/ Cell line	Route/ Dose	Activation	Results	References[b]	Comments
DNA damage	*B. subtilis* H17/M45	1 mg/disc (80% pure)	–	neg	Simmon *et al.*,1977	3 replicates. Same inhibition zone diameter between repair deficient and proficient strains.
DNA break	Col-E1 plasmid	1 Fg/ml in hexane (purity ns)	–	pos	Griffin & Hill, 1978	Incubation period ½ day to 4 weeks. DNA break elevated approx. 10-fold.
Mitotic Recomb.	*Sac. cerevisiae;* D3	50 mg/ml (80% pure)	±; m	pos	Simmon *et al.*, 1977	2 trials. Effects marginal; >3-fold increase in. the number of mitotic recombinants.

* Study acceptable for filing the SB950 data requirements.

a/ Abbreviations: UDS, unscheduled DNA synthesis; *S.*, *Salmonella*; *E.*, *Escherichia*; *B.*, *Bacillus*; *Sac.*, *Saccharomyces;* ns - purity not stated; +, with microsomal S9 fraction; -, without microsomal S9 fraction; m, mouse liver microsomal fraction S9.

b/ The reported dose was in mmol/kg. However, according to the study report, the specificity was 76 mCi/mmol. The administered 100 FCi should instead be 1.31 Fmol/kg.

Table 15. Effects of methyl parathion on *in vitro* induction of sister chromatid exchanges[a] .

Test[a]	Species/ cell line[b]	Route/ Dose[c]	Activation[d]	Results	References	Comments
SCE	V79	5, 20 Fg/ml (96.8% pure)	+; r	pos	Chen *et al.*, 1982	50 cells/dose, one trial. Significant effects at 20 Fg/ml.
SCE	V79; Human lymphoma B35M	10, 20, 40 Fg/ml (96.8% pure)	–	pos	Chen *et al.*, 1981	50 cells/dose, 2 replicates per cell line. Dose-related cell cycle delay was observed.
SCE	Human lymphoid LAZ007	0.02, 0.2, 2, and 20 Fg/ml (purity ns)[4]	±; r	pos	Sobti *et al.*, 1982	25 cells/dose, one trial. Significant SCE increase at 2, 20 Fg/ml. S-9 had no additional effect. Dose-related cell cycle delay noted.
SCE	Human lymphocytes	9.5, 19, 38 Fg/ml (purity ns)	–	pos	Singh *et al.*, 1984	30 cells/sample/dose, 10 samples. Dose-related delay of cell cycle.
SCE	Human lymphocytes	0.5, 1, 2, 3, 4, 5, 6, 9, 10, 13 ppm (purity ns)	_	pos	Gomez-Arroyo *et al.*, 1987	50 cells/dose, 2 replicates. Dose-dependent increase in SCE up to 4 ppm. Cell death occurred at 13 ppm.
SCE	Human lymphocytes	0.02. 0.04, 0.08, 0.16 Fg/ml	_	pos	Rupa *et al.*, 1990	positive at and above 0.04 Fg/ml after 48 and 72 hrs of treatment.

a/ Abbreviations: SCE, Sister Chromatid Exchange; V 79: Chinese hamster lung cell line V79; ns. purity not stated; ±, with and without microsomal S9 fraction; r, rat liver microsomal S9 fraction.

All six *in vitro* studies on sister chromatid exchange (SCE) (Table 15) showed positive results in the following mammalian cell lines: Chinese hamster lung cell V79, human lymphoma B35M and LAZ007, and human lymphocytes. Technically, SCE is an exchange of identical information between two chromatids. As such, the significance of a positive SCE test in risk assessment is less certain than other genotoxicity studies previously presented. SCE does not generally have good concordance with cancer bioassays. As a screening tool for oncogenicity bioassays, the reported specificity of SCE tests was 45% (Tennant et al., 1987).

11. REPRODUCTIVE TOXICITY

There was some indication that OPs in general may affect the menstrual cycle and cause an early menopause in humans. However, no data on human reproductive effects specifically to methyl parathion are available. There was some indication of correlation between serum LH and occupational exposures to three OPs (methyl parathion, ethyl parathion, methamidophos). Three multi generation reproductive toxicity studies in rats were submitted to DPR under the SB950 data requirement. One study was only in a summary form. Decreased pup survival was consistently found in all three studies. The search of the open literature revealed two reports showing ovarian toxicity in rats and one study showing possible sperm abnormalities in mice. Testicular and reproductive effects have also been reported in avian species.

11.1. Evidence in Humans

Reproductive effects from exposures to mixtures of OPs have been documented by Nakazawa and Nakazawa (1974) and Mattison (1985) among women in agriculture. These effects included abnormal menstruation (e.g., hypermenorrhea, oligomenorrhea, amenorrhea), and early menopause. On the other hand, Willis *et al*. (1993) found no effects of pesticide exposures (including methyl parathion) on the pregnancy outcome among 535 women enrolled in a southern California community clinic perinatal program.

No epidemiological data specific to methyl parathion alone are available. Padungtod *et al*. (1998) studied the profile of reproductive hormone (serum and urinary follicle-stimulating hormone, FSH; luteinizing hormone, LH; testosterone or its metabolite, urinary estrone conjugate E_1C) among 34 male Chinese factory workers, 20-40 years old. The subjects worked for at least 3 months in a factory that manufactured methyl and ethyl parathion, and methamidophos. The analysis showed that only the increase in serum LH was significantly correlated to pesticide exposures. The authors concluded that OP pesticides had a small effect on male reproductive hormones and suggested that the hormone disturbance might be secondary to potential testicular damage by OPs. A larger sample size is needed for detecting the relationship between OP exposure and serum testosterone or urinary E_1C level.

11.2. Multi Generation Studies in Rats

Three-generation study by Kronenberg *et al.* (1978)

Kronenberg *et al.* (1978) reported a 3-generation study conducted by Lobdell *et al.* (1966; as cited in Kronenberg *et al.*, 1978). However, an in-depth review of the study was not possible as the study was only available in a summary form. Rats were administered diets containing 0, 10, or 30 ppm methyl parathion. Effects reported at 30 ppm were: tremors in a few F_0 rats, reduced survival in weanlings of F_{1a}, F_{1b}, and F_{2a} groups, increased number of stillborn in F_{2b} and F_{3b}, and reduced reproductive performance in F_{2b} at the second mating, with only 41% of the rats having litters. Survival was also reduced (unspecified magnitude) in F_{3a} weanlings at 10 ppm. Therefore, the 10 ppm in the diet would be the LOEL. Alternately, the U.S. EPA (1988) identified 10 ppm in the diet as the No-Observed-Adverse-Effect-Level (NOAEL).

Two-generation Study by Daly and Hogan (1982)

The 2-generation study by Daly and Hogan (1982) was judged acceptable for filling the SB950 data requirement. Groups of 15 male and 30 female Sprague-Dawley rats were fed diets containing 0, 0.5, 5.0, or 25.0 ppm methyl parathion (93.6% pure) for 14 weeks before mating, and throughout the mating, gestation, and the lactation period. Many of the F_1 and F_2 pups had intestinal worms. The extent that the presence of worms may confound the evaluation of reproductive toxicity of methyl parathion may not be substantial because its occurrence was

Table 16. The maternal and pup body weight gain of Sprague-Dawley rats in a 2-generation study by Daly and Hogan (1982).

Conc. in diet (ppm)[a]	Maternal Body Weight gain (g)								Pup Body Weight gain (g)			
	Gestation day 0-20				Lactation day 0-21				Lactation day 0-21			
	F_0	% of control	F_1	% of control	F_0	% of control	F_1	% of control	F_1	% of control	F_2	% of control
0	116	100	129	100	19	100	19	100	37.7	100	34.1	100
0.5	121	104	124	96	18	95	17	89	40.5	107	33.2	97
5.0	121	104	123	95	18	95	14	74	34.6	92	33.3	98
25	123	106	113	88	-3**	(-16)	-7**	(-37)	32.9	89	30.6	90

**: Significantly different from control at p#0.01 (Dunnett's test)

a/ The respective doses at 5 and 25 ppm in the diet were approximately 0.4 and 2.3 mg/kg/day.

similar across all the treatment groups. No mortality occurred at any dose group. Marked reduction of the maternal body weight was evident at 25 ppm throughout the lactation period (Table 16). The reduction in pup body weight gain which was apparently dose-related, was not statistically significantly different from the controls. The survival of the F_2 pups at day 4 was statistically significantly lower (Dunnett's test; p#0.05) at 25 ppm. The respective survival rates
from the controls to the highest dose group were: 258/263 (98.1%), 280/284 (98.6%), 219/223 (98.2%), and 199/213 (93.4%). Based on the survival of the pups and the maternal weight gain reduction, the reproductive toxicity and maternal toxicity NOEL of 5 ppm was determined. Estimated from the data on body weight and food intake during the weeks before mating, the dose was approximately 0.4 mg/kg/day at 5 ppm and 2.3 mg/kg/day at 25 ppm in the diet. No data were available for calculating the dose during the gestation and lactation periods.

Although this study was conducted for testing the reproductive toxicity of methyl parathion, the histopathological examination revealed adrenal cortex adenomas in two of the 10 F_0 male rats in the 25 ppm dose groups. The significance of these findings was previously discussed in Section 9.1., *Oral Studies in Rats.*

Three-generation study by Loser and Eiben (1982)

In a 3-generation reproductive study by Loser and Eiben (1982), groups of 10 male and 20 female Wistar rats were fed diets containing 0, 2, 10, or 50 ppm methyl parathion (95% pure). One rat from each group of female control, female 10 ppm, and male 50 ppm died. According to the report, the death was not attributed to the treatment. In litters with more than 10 pups, the number of pups was culled to 10 pups per litter 5 days after birth. Data on ChE activity and clinical observations were not available. As shown in Table 17, significant reduction in pup survival up to day 5 and at the end of week 4 consistently occurred at 10 and 50 ppm. Maternal body weight and behavior were not affected at the dose range tested. Pup weight reduction and growth retardation occurred at 50 ppm. Based on the reduction of pup survival at 10 ppm, a reproductive toxicity NOEL of 2 ppm was determined. The report lacked a quality assurance statement, diet and test article analysis, food consumption measurements, complete clinical observations, necropsy data, and proper body weight measurements. Without these data, an estimation of dose was not possible. Based on the dietary patterns of Wistar rats as reported in the chronic study by Bomhard *et al.* (1981), the respective doses at 2 and 10 ppm were estimated as 0.14 and 0.71 mg/kg/day for the females (see: Section 8.1.).

11.3. Male and Female Reproductive Toxicities in Rodents

Mathew *et al.* (1992) used a sperm abnormality assay to evaluate the toxicity of methyl parathion. Increases in the percentage of abnormal sperms were reported in groups of 5 mice, 1 and 5 weeks after receiving a single oral administration of 9.375, 18.75, 37.50, or 75.00 mg/kg Metacid 50 (manufactured in India, purity not specified). The abnormalities included:

Table 17. Pup survival from a 3-generation reproductive toxicity study in Wistar rats by Loser and Eiben (1982).

Conc. in diet[a]	Pup survival rates (% of ave. pups/litter at birth) F1a	F1b	F2a	F2b	F3a	F3b
Survival to Day 5						
0 ppm	97.8	97.7	94.1	99.0	93.7	91.9
2 ppm	99.4	95.7	99.0**	89.9**	99.3*	98.1
10 ppm	93.4	84.9**	96.8	87.4**	94.7	89.5
50 ppm	22.1**	27.1	18.2**	0**	-	-
Survival to Week 4[b]						
0 ppm	100	98.1	98.9	92.4	84.5	90.8
2 ppm	99.4	96.3	98.4	87.4	97.9**	97.2*
10 ppm	95.5**	95.7	92.9**	83.2*	96.7**	95.3
50 ppm	44.4**	42.9**	50.0**	-	-	-

Levels of statistical significance as compared to the controls: * for p#0.05; ** for p#0.01.
a/ The respective doses at 2 and 10 ppm in the diet were approximately 0.14 and 0.71 mg/kg/day.
b/ For litters that had more than 10 pups, the number was reduced to 10 per litter on day 5.

amorphous, hookless, banana, folded, and double-headed/tailed sperm. The percentage of abnormalities was greater when scored at 5 weeks than 1 week after dosing with the mean of 12.3% at the highest dose. The implication of the effects with respect to the reproductive outcome is not known. Moreover, the dose levels were much higher than the levels used in rat studies and appeared to be at or exceeded the range of the LD_{50} for mice (Table 1). According to the report, however, the dose levels were 1/16 - ½ of the LD_{50} and no signs of acute toxicity were mentioned. The lack of detail in reporting precludes further evaluation of this report for hazard identification. No other studies are available in the literature for evaluating the potential of methyl parathion in causing sperm morphological abnormalities.

Ovarian effects of methyl parathion in hemicastrated rats (right ovary removed) were recently published in two reports by Kaliwal and his coworkers. Hemicastration significantly increased both the ovarian weight with approximately 45% compensatory hypertrophy, and the number of healthy and atretic

follicles. In one report by Dhondup and Kaliwal (1997), groups of at least 6 Wistar rats, 80-100 days old, were administered methyl parathion (metacid, 50% purity) intraperitoneally at 0, 2.5, 3.5, 4.0, and 5.0 mg/kg/day for 15 days. There was a dose-related increase in the length of estrous cycles from 4.77 days/cycle to 5.0, 9.0, 12.9, and 15 days/cycle from the low to the high dose groups. On the day of sacrifice (day 16 of treatment), all rats treated with methyl parathion were in diestrus while the controls were in proestrus, estrus, or metestrus. In addition to the prolonged estrus cycles, rats at 4.0 and 5.0 mg/kg/day also had reductions in the body weight gain and relative uterus weight, the compensatory hypertrophy, and the number of healthy follicles. Respectively for these two dose groups, body weight gains were 88 and 49% of the control. The index of ovarian compensatory hypertrophy (OCH) was calculated as the ratio (in percentage) of the relative ovarian weight between the treatment groups to the sham controls. Compared to the OCH of 45% in the hemicastrated controls, the OCH at 4

and 5 mg/kg/day were only 13 and 8%, respectively. The number of healthy follicles in these two high dose groups were 76 and 70% of the controls. The mechanism for these ovarian effects and alteration of estrus cycles was unknown but the authors noted that these effects did not appear to be directly correlated with the body weight reduction.

Similar results were reported by Asmathbanu and Kaliwal (1997) in groups of 4-6 hemicastrated female Wistar rats, 80-100 days old, received 5 mg/kg/day methyl parathion (metacid, 50% pure) through i.p. injection for 1, 5, 10, and 15 days. Body weight gain and the relative uterus weight showed a decline throughout the 15 days and were respectively 74% and 78% of the controls after 15 days of treatment, although the difference in the body weight gain were not statistically significant. The number of healthy follicles were 84% and 69% of the controls after 10 and 15 days. The number of normal estrous cycles and the duration of estrus and diestrus were prolonged while the duration for metestrus was shortened.

11.4. Studies in Avian Species

Solecki *et al*. (1996) studied the effects of methyl parathion on the reproduction of Japanese Quail. Male and female birds were fed diets containing 0, 3, 12, or 48 ppm methyl parathion (93.1% pure) and mated for 6 weeks. While no clinical symptoms and behavior changes were observed, statistically significant brain AChE inhibition was noted at all treatment groups (approximately 20% inhibition at 3 ppm). Reproductive effects were noted at 48 ppm. These included the reduction in the number of eggs laid (~20% reduction), egg weight (~9% reduction), and eggshell thickness (7-10% reduction).

Maitra and Sarkar (1996) studied the reproductive effects of methyl parathion in male white throated munias from natural population. Birds received a single oral intubation of 5, 10, or 20 Fg/100 g/day methyl parathion were sacrificed on day 1, 5, or 10 and evaluated for AChE activities (brain and testes) and histopathologically (testes). Dose-related brain AChE inhibition was statistically significant (p#0.05) at 10 and 20 Fg/100 g on day 1 (approximately 30% inhibition) and at all dose levels on day 5 and 10 (up to 50% inhibition). Testicular AChE inhibition was statistically significant only at 10 and

20 Fg/100 g after 10 days of dosing (approximately 50% inhibition). Dose-related changes included decreases in the percentage of tubules with healthy germ cells (up to approx. 40%) starting day 1, the seminiferous diameter (up to approx. 50% reduction) starting day 5, and the testicular weight (up to approx. 40%) on 10 days of dosing.

12. DEVELOPMENTAL TOXICITY

The embryo-, fetal-, and developmental toxicity of methyl parathion have been studied in rats, rabbits, mice, and avian species. Among the effects reported in rats and rabbits were: lower fetal body weight, increased resorption, reduced pup survival, and abnormalities and variations of ossification. There was also some indication of neurobehavioral effects in rat pups that received *in utero* exposures. In addition to a lower fetal body weight, mice fetuses that received *in utero* exposures at much higher dose levels also had cleft palate and increased death. Injection of methyl parathion into the air space of chicken eggs resulted in cervical lordosis and scoliosis, cervical muscle atrophy, lower body weight, and retarded growth.

12.1. Studies in Rats

In a study by Fish (1966), groups of 2-3 Holzmann rats received a single intraperitoneal (i.p.) injection of 4 or 6 mg/kg methyl parathion on day 9 or 15 of gestation and were killed on day 21. Fetuses from rats that received 4 mg/kg on gestation day 15 had lower body weight (81% of the controls). A reduction of the maternal RBC ChE activity (values not given) was noted in all treated rats at 4 and 20 hours after dosing. Tanimura *et al.* (1967) also reported lower fetal body weight (90% of the controls) in rats that received a single i.p. injection of 15 mg/kg of methyl parathion at on day 12 of gestation. The effect was not observed at 5 or 10 mg/kg.

In a study by Crowder *et al.* (1980) groups of 3 Sprague-Dawley rats received 0 or 1 mg/kg/day methyl parathion (99.9% pure), via gavage, on day 7 through day 15 of gestation. An increase in pup mortality up to day 15 postpartum was reported (30% in the treated group versus 10% in the controls). The results of neurobehavioral tests showed that neonates of dams that received 1 mg/kg/day methyl parathion had slightly shorter grasp-hold time in the reflex test (approximately 30% reduction in hold time for 15 days old pups, as estimated from a figure in the report). The open field test also showed that pups between 25 and 55 days old from the treatment groups covered a greater area of movement. Methyl parathion treated rats tested at 55 days old also showed some compromised ability to transfer knowledge (i.e., switching direction from right to left) in the maze test. Based on the post-partum survival of pups and the behavioral effects, 1 mg/kg/day was the LOEL.

Gupta *et al.* (1985) also reported neurobehavioral effects in Wistar-Furth rats at the same dose level (1 mg/kg/day) used in the above study by Crowder *et al.* (1980). Pregnant rats ($12, number not

specified) were orally administered methyl parathion at 1.0 or 1.5 mg/kg/day on day 6 through day 20 of gestation. The results showing positive effects are summarized in Table 18.

Muscle fasciculations and tremors occurred in the dams after 3 to 4 days of exposure 1.5 mg/kg/day, beginning 15-30 minutes after dosing and lasted for 2-4 hours. Maternal body weight gain reduction and increased late resorption also occurred at 1.5 mg/kg/day. Dose-related decreases of ChE activities and a concomitant increase in the activity of choline acetyltransferase (an enzyme catalyzing the synthesis of ACh) were also detected in the brain cortex of dams on day 19 of gestation. The inhibition of ChE was detected in the offspring from both treatment groups on postnatal days 1, 7, 14, 21 and 28 at some or all 4 brain regions examined (i.e., frontal cortex, brain stem, striatum, and hippocampus). Data on day 1 and 14 are provided in Table 18. In addition to ChE inhibition, prenatal exposure resulted in behavioral alternations at 1 mg/kg/day. These included reduced accommodated locomotor activity at 2 months of age and decreased latency in cage emergence and changes in operant behavioral (bar pressing patterns) at 3 months of age (Table 18). However, the cage emergence test showed a slightly prolonged, instead of shortened, emergence time at 1.5 mg/kg/day. The maternal NOEL based on the overt clinical signs was 1 mg/kg/day. However, the lowest tested dose of 1 mg/kg/day was the LOEL based on brain ChE inhibitions in the dams and pups. This LOEL was also supported by the indication of neurobehavioral effects.

Two rat teratology studies (Machemer, 1977; Becker *et al*., 1987) are on file at DPR. In a study by Machemer (1977), groups of 20 Wistar rats received 0, 0.1, 0.3, or 1.0 mg/kg/day methyl parathion (94.4% pure), via gavage, on days 6 through 15 of gestation. The maternal body weight gain during the treatment period was significantly ($p<0.01$) lower (43% reduction) at 1.0 mg/kg/day. A 5% reduction of fetal body weight ($p<0.05$) also occurred at this dose level. The study was deficient in lacking dosing solution analysis, individual data on body weight, food consumption data, necropsy parameters, fetal examinations, fetal body weight, and clinical observations. Based on the reduction of body weight gain, the maternal and fetal NOEL was 0.3 mg/kg/day.

A study by Becker *et al.*, (1987) was judged acceptable in filling the SB950 data requirement for tests conducted in rats. In this study, groups of 25 pregnant Wistar/HAN rats were administered 0, 0.3, 1.0, or 3.0 mg/kg/day methyl parathion (97% pure) via gavage, on days 6 through 15 of gestation. The activities of ChE were monitored in 10 additional rats at 0 and 3.0 mg/kg/day. The results are summarized in Table 19. The maternal plasma, RBC, and brain ChE were significantly inhibited at 3.0 mg/kg/day on gestation day 16 (one day after the last dosing). A definitive NOEL for the brain ChE inhibition cannot be established because ChE activities were not measured in the low and mid dose groups. Cholinergic signs were not reported in the dams at 3.0 mg/kg/day until day 8 of treatment. However, death (5 of 35 dams; 14.3%) occurred beginning day 7. The cholinergic signs included: somnolence, ataxia, dyspnea, salivation, ventral recumbency, repeated chewing and occasional whining. The food consumption was reduced by approximately 8% at the same dose. Substantial body weight gain reduction was noted at 3 mg/kg/day. Excluding the uterus weight, the adjusted body weight

Table 18. Maternal and neonatal toxicity of methyl parathion in Wistar-Furth rats (Data from Gupta *et al*., 1985)[a]

Effects		Controls	1 mg/kg/day	1.5 mg/kg/day
Maternal				
Brain Cortex ChE (gestation day 19)		100±2.9%	79±3.73%*	40±7.5%*
Brain Cortex CAT[b] (gestation day 19)		10.03±0.17	13.07±0.74*	15.25±1.00*
Weight gain (gestation day 15)		16%	-	11%
Muscle fasciculations, tremors (began after 3-4 days of exposure)		negative	negative	positive[c]
Resorption		0%	-	25%
Pup (postnatal day 1)				
Frontal Cortex ChE		(100%)	63.8±4.0*	55.3±6.9*
Brain Stem ChE		(100%)	82.6±1.6*	52.4±3.5*
Pup (postnatal day 14)				
Frontal Cortex ChE		(100%)	98.0±4.1	59.0±4.9*
Brain Stem ChE		(100%)	83.9±4.9*	62.8±6.7*
Striatum ChE	(100%)	86.1±4.1*	58.9±5.1*	
Hippocampus ChE	(100%)	99.8±4.5	52.4±3.7*	
Postnatal behavioral tests				
2 months: Accommodated locomotor activity (counts)		133±10	118±9*	100±13
3 months: Cage emergence (sec)		598±144	71±31*	720±120
3-6 months: Operant behavior (sec)[d]		6.8	9.5*	-

a/ Methyl parathion treatment on day 6 through day 20 of gestation. Values for ChE activities in the pups were from 5 to 7 litters (2 rats/liter). Values for behavioral tests were from 8 to12 animals.

b/ CAT: choline acetyltransferase; values in nmol acetylcholine synthesized per hour per mg protein.

c/ Incidence data were not available.

d/ Behavior patterns included the latency to bar press and number of days to an asymptotic rate of bar pressing during acquisition. Values estimated from a bar graph.

* Statistical significance at $p<0.05$.

Table 19. Maternal and fetal toxicity of methyl parathion in Wistar-HAN rats (Data from Becker *et al.*, 1987)[a]

	Dose (mg/kg/day)			
	0	0.3	1.0	3.0
Maternal ChE (gestation day 16)				
plasma	(100)	-	-	59%**
RBC	(100)	-	-	29%**
brain (100)	-	-	78%**	
Maternal Death (day 7-10)	0% (0/35)	0% (0/25)	0% (0/25)	14.3% (5/35)
Maternal Body Weight gain (% of gestation day 6)				
gestation day 8	1.8%	1.7%	1.3%	1.8%
gestation day 10 5.3%	4.8%	4.8%	2.7%**	
gestation day 12 9.6%	8.3%	8.8%	4.9%**	
gestation day 14 13.6%	12.7%	12.3%	4.9%**	
gestation day 15 15.4%	14.8%	14.0%	5.4%**	
gestation day 16 19.7%	18.3%	17.5%	6.2%**	
gestation day 21 43%	41.9%	40.8%	28.9%**	
Fetal Body weight (g)	4.8±0.1	4.9±0.2	4.8±0.2	4.4±0.5**
Resorption				
number of fetus per litter	15/24 (0.6)	18/24 (0.8)	16/24 (0.7)	20/20 (1.0)
number of litter affected	10/24 (42%)	12/24 (50%)	12/24 (50%0	11/20 (55%)
Fetal Ossification				
incomplete: Cranium (occipital)	1/156 (1%)	- -	- -	12/125 (10%)
non-ossified: cervical vertebrate 1-4	2-13%	4-23%	-	6-26%

a/ Pregnant rats were treated during day 6 through day 15 of gestation.

** Statistically significant at $p<0.01$

gain throughout the gestation period was 7.0 gm in the controls while dams at 3.0 mg/kg/day had a weight loss of 3.9 gm. Fetal effects, including 8% lower fetal body weight (statistically significant at $p<0.05$) and delayed ossification, also occurred at 3.0 mg/kg/day (Table 19). Although not statistically significant, the incidence of resorption appeared to be dose-related. The maternal and fetal NOEL was 1.0 mg/kg/day.

In a study by Kumar and Devi (1996), groups of 10 Wistar rats received 0, 0.5, 1, and 1.5 mg/kg/day methyl parathion (98% pure) by gavage on day 6 through day 20 of gestation. Maternal and fetal effects were noted at 1.5 mg/kg/day. Maternal toxicities included muscle fasciculations, tremors, prostration, mild clonic convulsions, day 0-19 body weight gain reduction (79% of controls), and reduction of the weight of placenta (53% of controls) and amniotic fluid (80% of control). Fetal body weight at 1.5 mg/kg/day was lower (77% of controls). The increase in resorption appeared to be dose-related (0%, 5%, 8%, 49% from controls to high dose groups) and was statistically significant at 1.5 mg/kg/day. Fetal skeletal anomalies include incomplete bipartite or missing sternebrae, extra or rudimental ribs, and incomplete ossification.

12.2. Studies in Rabbits

Two studies (Renhof, 1984; Hoberman, 1991) are on file at DPR. In a study by Renhof (1984), groups of 12-15 inseminated Himalayan rabbits were administered 0, 0.3, 1.0, or 3.0 mg/kg/day methyl parathion orally on days 6 through 18 of gestation. No embryotoxicity, teratogenicity, or maternal toxicity was found in this study.

A later study by Hoberman (1991) was judged acceptable for filling the SB950 data requirement for tests conducted in rabbits. In this study, groups of 19-20 artificially inseminated New Zealand White rabbits received 0, 0.3, 3.0, and 9.0 mg/kg/day methyl parathion (95.7% pure) by gavage on gestation days 6 through 18. The reduction in maternal RBC ChE activity was statistically significant ($p<0.05$) at all levels of methyl parathion (the respective inhibitions from the low to high dose groups were 18%, 43%, and 75%). The inhibition of plasma ChE was statistically significant ($p<0.01$) only at 9.0 mg/kg/day (at 50% inhibition). No clinical signs of toxicity were observed in the dams during the study. Neither were there any abnormal findings at necropsy. An increased incidence (4 in 141 fetuses) of thickened areas of ossification in the ribs was noted in the fetuses at 9.0 mg/kg/day. The lowest dose of 0.3 mg/kg/day was the maternal LOEL based on a statistically significant RBC ChE inhibition. However, the toxicological significance of 18% RBC ChE inhibition was uncertain without the report of any clinical signs of toxicity. The NOEL for fetal effects was 3.0 mg/kg/day.

12.3. Studies in Mice

Tanimura *et al*. (1967) reported developmental effects that were more prominent than what was observed in the aforementioned studies, albeit at a much higher dose range. Cleft palate, increased mortality, and reduced fetal body weight were observed in 13 of the 112 fetuses from mice that received an i.p. injection of methyl parathion at 60 mg/kg on day 10 of gestation. These effects were not found at 20 mg/kg. The dose range applied in this study appeared to exceed the i.p. LD_{50} values compiled in Table 1 (between 8.2 and 11.0 mg/kg). Instead, the report stated that the LD_{50} was near the high dose level. Severe acute toxicities, including ataxia and convulsions, were evident in all treated mice within 30 minutes of dosing. As expected, death occurred to some mice at the high dose.

12.4. Studies in Avian Species

A single injection of Wofatox 50 EC (50% methyl parathion emulsifiable concentrates) into the air space of embryonated chicken eggs at 16 and 160 mg/kg and pheasant eggs at 13.5 - 270 mg/kg on day 12 of incubation resulted in cervical lordosis (forward curvature of the spine) and scoliosis (sideway curvature of the spine), and atrophy of the cervical muscles (Varnagy *et al.*, 1984; Varnagy and Deli, 1985). Deli and Kiss (1988) also noted a decrease in cellular content of cytoskeletal proteins (a- and ß-tubulin and a-actinin) in the cervical muscles of embryos that received 20 mg/day methyl parathion injections (0.5 ml of 0.4% solution) for 4 or 8 days. The reduction in protein contents may have contributed to the muscular atrophy. Kumar and Devi (1992) reported teratogenic signs in developing chicken at much lower dose levels. Chicken embryos were treated with 0, 5, 10, or 50 Fg methyl parathion (in groundnut oil) via a yoke sac route on days 4, 6, and 9 of incubation and sacrificed on day 20. The body weight of the embryos that received 10 and 50 Fg methyl parathion was significantly ($p<0.05$) lower (78-83% of the controls). Embryo viability was also reduced at these dose levels. Growth retardation was evident in the shorter body length and leg bones. Teratological effects at these levels included shorter necks, leg muscle hypoplasia, abdominal hernias, and hemorrhagic spots in the brain and upper body.

13. NEUROTOXICITY

Studies which specifically investigated the neurotoxicities of methyl parathion are presented in this section. Two studies (Schulz *et al*., 1990; Kumar and Desiraju, 1992) in Wistar rats were available in the open literature, however, no NOEL could be directly determined from these studies. The study by Kumar and Desiraju (1992), which contained data of both acute and subchronic toxicities, is presented under the following Section 13.1., *Acute Neurotoxicity*. Two relatively extensive studies by Minnema (1994a, b) in Sprague-Dawley rats were submitted to DPR in October 1998.

13.1. Acute Neurotoxicity

Kumar and Desiraju (1992) studied the ChE inhibition in 7 regions of the central nervous system in young Wistar rats: cerebellum, motor cortex, hippocampus, hypothalamus, striatum accumbens, ventral brain stem, and spinal cord. Female pups received oral doses of methyl parathion for a number of days: 1-day (1 mg/kg on postnatal day 15), 15-day (0.1 mg/kg from postnatal day 2), or 150-day (0.2 mg/kg/day from postnatal day 2). ChE activities were measured in 3 to 6 rats per dose group. The number of affected CNS regions appeared to increase with the length of the dosing period. The ChE activity was inhibited in the brain stem, motor cortex and cerebellum, beginning at the first measurement interval at 20 minutes after a single exposure (15-25% inhibition). The peak inhibition was approximately 55% at 120 minutes after the exposure. Under the same single dosing regimen, adult (70 days old) rats also showed similar response (approximately 55-60% inhibition) in these 3 regions. Two weeks of dosing resulted in ChE depressions in the brain stem and spinal cord (ChE level not given in the report). The 150-day exposure at 0.2 mg/kg/day starting on postnatal day 2 resulted in at least 24% ChE depression in 5 regions (all but cerebellum and motor cortex). In addition to the changes in the ChE activities, the preliminary study showed that a single exposure of 2.5 mg/kg to rats at postnatal day 15 resulted in death of all pups (number not given). Cholinergic signs ("shivering", salivation, muscular fasciculation) occurred at 1 mg/kg/day, started 15-20 minutes after dosing and lasted for approximately 2 hours. No NOEL can be determined from this study. Based on the brain ChE inhibition and clinical observations, the 1 mg/kg/day was the acute LOEL. Based on the brain ChE inhibition, the 0.2 mg/kg/day was the subchronic LOEL.

An acute neurotoxicity study by Minnema (1994a) is on file at DPR. In this study, groups of 7-8 weeks old male and female Sprague-Dawley Cr1:CD BR rats were given a single gavage administration of methyl parathion (93.1% purity) at 0, 0.025, 7.5, or 10.0 (males) and 15 (females) mg/kg/day. Groups of 10 rats per sex were subject to neurobehavioral tests while groups of 5 rats per sex were assigned for ChE activity determination. Locomotor activity and Functional Observational Battery (FOB) that measured the various aspects of sensory and motor functions were conducted at 1.5 hours, and 1 and 2 weeks after dosing. ChE activities were measured at 1.5 hours (for all dose groups) and 2 weeks (for the controls and the high dose groups) after dosing. The neurobehavioral tests included home-cage and hand-held observations, open-field observations (arousal, circling, gait, posture, stereotypy, tremors, convulsions, and other signs), response observations (light approach, catalepsy, olfactory, pupil, righting reflex, touch, and others), performance measures (grip strength, foot splay, tail flick, and body temperature), and automated auditory startle response. Paraffin- or plastic-embedded nerve tissues were examined histopathologically. Initially, the examination was conducted for the controls and the high dose groups. When abnormalities were found at the higher dose, tissues from the next lower dose(s) were then examined.

The effects of methyl parathion were summarized in Table 20. Salivation, hypoactivity, muscle fasciculations, ataxia, and tremors were observed at 7.5, 10, and 15 mg/kg/day. In addition, red perinasal crust, chromodacryorrhea, respiratory distress, urine stains, and death were also reported for male and female rats at 10 and 15 mg/kg/day within 2 days of dosing. The reduction of mean body weight gain during day 0 to 7 was statistically significant in the males (49% lower than the controls) at 10 mg/kg/day. Additionally, rats at these dose groups also showed changes in the neurobehavioral parameters within the FOB. These included limp handling/body tone, flattened posture, gait, arousal and hypoalertness, rearing, absence of pupil response, righting reflex, reduced rectal temperature, and fore- and hind-limb grip strength. No effects were reported at the lowest dose. No significant FOB were noted 1 or 2 weeks of the exposure. Focal demyelination showing small foci of myelin vacuolation and fragmentation in the peripheral nervous system, with occasional glial cells associated with the foci were found in lumbar dorsal and ventral root fiber, tibial, and proximal sciatic nerves. The mechanism for demyelination is not understood. These effects were reported in the study as treatment-related at 7.5, 10, and 15 mg/kg/day, secondary to axonal damages common in aging rats. Nevertheless, it should be noted that the rats used in this study were 7-8 weeks old. The report considered that the observations at the controls and low dose were spontaneous. Based on the clear effects of demyelination at and above 7.5 mg/kg, the NOEL was 0.025 mg/kg. However, it should be noted that there was an apparent slight increased incidence of demyelination in the lumbar root fibers at 0.025 mg/kg.

Data on ChE activities were also given in Table 20. The ChE activities in the plasma, RBC, and various regions of the brain were severely inhibited at 7.5, 10, and 15 mg/kg/day. The RBC and brain ChE at the high dose groups showed substantial recovery by day 14 of dosing, albeit remained to be statistically significantly lower than the controls. The ChE activities were not measured for the low and mid dose groups on day 14. The NOEL based on plasma, RBC and brain ChE inhibitions was 0.025 m/kg/day, although there were a 22% plasma ChE inhibition at this dose level.

13.2. Subchronic Neurotoxicity

In the study by Schulz *et al.* (1990) mentioned in Section 6,3., *Thresholds for Acute Toxicity*, the neurobehavioral effects of methyl parathion were evaluated in groups of 20 male Wistar rats after receiving 0, 0.22, or 0.44 mg/kg/day methyl parathion (60% pure, impurities unknown) via gavage, 5 days per week, for six weeks. Behavioral effects were evaluated with the open-field (OF) and elevated plus-maze (EPM) tests. The authors considered a number of indices as demonstrating significant effects of methyl parathion. Based on the data presented, the more noticeable effects included: increased urination, reduced grooming, prolonged latency both for leaving the center field and for rearing (from the OF tests), and decreased defecation (from the EPM tests). These effects were more definitive at 0.44 mg/kg/day. Further interpretation of the behavioral effects cannot be made due to the limited information.

Table 20. Effects of methyl parathion in Sprague-Dawley Crl:CD BR rats after a single gavage administration[a].

Effects	Dose (mg/kg)							
	Males				Females			
	0	0.025	7.5	10	0	0.025	7.5	15
Clinical Observations - Number of rats affected per 10 rats								
Salivation	0	0	8	5	0	0	8	8
Hypoactivity	0	0	9	6	0	0	8	7
Muscle fascicul.	0	0	5	6	0	0	7	8
Ataxia	0	0	8	6	0	0	4	3
Tremors	0	0	10	6	0	0	9	8
Death	0	0	0	3	0	0	0	3
ChE Activities[b] - % of Controls (average of 5 rats per group)								
Plasma	100	78	35*	25*	100	91	29*	24*
RBC	100	101	44*	44*	100	100	43*	42*
Brain								
CTX	100	97	12*	7*	100	97	18*	10*
CBL	100	104	18*	9*	100	108	21*	12*
HIP	100	101	14*	7*	100	100	21*	9*
STR	100	98	10*	5*	100	107	15*	7*
OLB	100	101	14*	9*	100	105	23*	10*
BRS	100	99	14*	7*	100	98	24*	10*
Demyelination of Nerves - Number of rats affected per 6 rats								
Lumbar; root fibers								
dorsal	0	3	4	5	1	-	0	3
ventral	2	3	4	4	1	-	0	3
Tibial	0	0	1	3	1	-	0	1
Proximal sciatic	1	-	1	3	1	-	0	1
Sural	0	-	0	2	0	-	0	0

a/ Data from Minnema, 1994a.

b/ ChE measured 1.5 hours after a single gavage administration of methyl parathion. Brain regions: CTX: Cortex; CBL: Cerebellum; HIP, hippocampus; STR, striatum; OLB, olfactory bulb; BRS: brainstem. Levels of statistical significance as compared to the controls was reported only at p#0.05, marked by *.

A subchronic neurotoxicity study by Minnema (1994b) is on file at DPR. The study protocol was similar to the acute neurotoxicity by the same author (Minnema, 1994a), except that methyl parathion was administered through dietary inclusion instead of gavage. In this study, groups of 7 weeks old male and female Sprague-Dawley Cr1:CD BR rats received diets containing methyl parathion (93.1% purity) at 0, 0.5, 5, or 50 ppm for 13 weeks. The respective dose at 0.5, 5, and 50 ppm, calculated based on food consumption and body weight data over the period of the study, were 0.029, 0.29, and 2.9 mg/kg/day for the males and 0.037, 0.37, and 4.2 mg/kg/day for the females. Groups of 10 rats per sex were subject to neurobehavioral tests while groups of 5 rats per sex were assigned for ChE activity determinations on week 4, 8 and 13 (neurobehavior tests) or week 14 (ChE measurements). Additional groups of 5 controls and high dose rats were kept beyond the 13 weeks of dosing for a study on recovery (week 16 for neurobehavior tests and week 17 for ChE activity measurements). The once a day cageside observation was performed in the morning. FOB that measured the various aspects of sensory and motor functions were conducted during the dark cycle. It included home-cage and hand-held observations, open-field observations (arousal, circling, gait, posture, stereotypy, tremors, convulsions, and other signs), response observations (light approach, catalepsy, olfactory, pupil, righting reflex, touch, and others), performance measures (grip strength, foot splay, tail flick, and body temperature), and automated auditory startle response. Paraffin- or plastic-embedded nerve tissues from the controls and the high dose groups were examined histopathologically.

The results were summarized in Table 21. Increased incidence of alopecia and skin sore were observed at all dose levels. Other clinical effects reported at 50 ppm included urine stain, hunched posture, tremors, having thin appearance and chromodacryorrhea. A female rat had a small mass in the cervical region. Decreased food consumption and body weight, increased incidence of neurobehavioral effected were additionally reported at 50 ppm. The neurobehavioral effects included latency to first step (3.2 - 6.9 seconds, compared to 1.0 - 1.8 seconds in the controls), absence of pupil response to approaching penlight from each side of head (1 male and 4 females on week 4), other pupillary responses, reduced fore-limb and hind-limb grip strength (up to 30% lower than the controls on week 4), and tremors (2 of 10 females). No remarkable axonal degeneration was reported at 50 ppm (2.9 - 4.2 mg/kg/day). However, it should be noted that demyelination in lumbar root fibers, tibial, peroneal, and proximal sciatic nerves was reported by the same author in rats that received a single exposure at or above 7.5 mg/kg (Minnema, 1994a) and by Daly (1991) in rats after 12 months of exposure to 2.5 ppm methyl parathion in the diet (0.1 mg/kg/day) (see Section 8.1., under CHRONIC TOXICITY). Data on ChE activities were also given in Table 21. Brain ChE activities were severely inhibited at 50 ppm. The RBC ChE activities were lower by 23-28%, which was statistically significant. The NOEL was 5 ppm (0.29-0.37 mg/kg/day) based on the decreased body weight gain and food consumption, and clinical and neurobehavioral effects occurred at 50 ppm. It should be noted that increased alopecia and skin sore occurred at all dose levels (0.5 - 50 ppm, or 0.029 -4.2 mg/kg/day) including at and below the NOEL. The NOEL was 0.5 ppm (0.029-0.037 mg/kg/day) based on the RBC ChE inhibition at 5 ppm.

Table 21. Effects of methyl parathion in Sprague-Dawley Crl:CD BR rats after 13 weeks of treatment through the diets[a].

Effects	Concentrations in the diet (ppm)[b]							
	Males				Females			
	0	0.5	5	50	0	0.5	5	50
Clinical Observations - Number of rats affected per 15 rats								
Skin/Pelage								
alopecia	0	3	2	7	1	3	1	6
skin sore	0	0	1	2	0	1	3	1
Urine stain	0	0	0	0	0	0	0	1
Hunched posture	0	0	0	0	0	0	0	1
Tremors	0	0	0	0	0	0	0	2
Body Weight - g								
week 7	488	489	491	447	264	264	256	247
week 13	571	577	580	538	305	298	305	287
Food Intake - g/week								
week 7	190	189	191	183	133	135	129	145
week 13	194	184	190	174	122	114	116	127
ChE Activities[c] - % of Controls (average of 5 rats per group)								
Plasma	100	111	97	39*	100	101	94	15*
RBC	100	102	72*	48*	100	99	77*	45*
Brain								
CTX	100	96	96	39*	100	123	98	18*
CBL	100	103	103	62*	100	103	98	35*
HIP	100	95	94	37*	100	104	93	13*
STR	100	88	95	25*	100	101	86	7*
OLB	100	97	98	39*	100	106	110	17*
BRS	100	114	110	45*	100	101	99	22*

a/ Data from Minnema, 1994b.

b/ The respective average dose at 0.5, 5, and 50 ppm, calculated based on food consumption and body weight data, were 0.029, 0.29, and 2.9 mg/kg/day for the males and 0.037, 0.37, and 4.2 mg/kg/day for the females.

c/ ChE data were those measured 14 weeks after the onset of a 13-week dietary exposures. The Plasma and RBC ChE inhibitions were slightly lower on week 4 and 8. Brain regions: CTX: Cortex; CBL: Cerebellum; HIP, hippocampus; STR, striatum; OLB, olfactory bulb; BRS: brainstem. Statistical significance as compared to the controls was reported only at p#0.05, marked by *.

14. DELAYED NEUROPATHY

Some OPs cause degenerative changes in nerves, a process known as organophosphorus-induced delayed polyneuropathy (OPIDP or OPIDN). Instead of the mechanism of AChE inhibition, OPIDN is associated with the inhibition of neuropathy target esterase or neuropathy target enzyme (NTE). The exact mechanism is not yet understood. Histopathologically, OPIDN is axonopathy of central-peripheral distal sensory-motor nerves (Lotti, 1992). Clinical manifestation in humans is commonly characterized by flaccid paralysis of lower limbs, although upper limbs are also involved in severe cases. These signs are generally not apparent until 2-3 weeks after the exposure. Results of investigations specific for methyl parathion are presented in this section. The available studies showed no evidence that methyl parathion causes acute delayed neuropathy. Neuropathy occurring after chronic exposures was presented in the Chronic Toxicity (Section 8., *CHRONIC TOXICITY*).

Barnes and Denz (1953) reported no paralysis in hens that received a methyl parathion injection at an unspecified dose that was sufficient to produce a cholinergic response. Gaines (1969) studied the neurotoxicity of 9 carbamates and 30 OPs, including methyl parathion. In hens which were pre-treated with 15 mg/kg atropine and received a subcutaneous injection of 64 mg/kg methyl parathion, leg weakness was observed within 24 hours after dosing. The recovery was complete within 28 days. No effects were observed at 32 mg/kg. In a later study by Ohkawa *et al*. (1980), 5 to 10 hens (given atropine as needed) were given a single oral dose of 100 mg/kg methyl parathion and kept for 4 weeks for examination. At day 2 after the treatment, neurotoxic esterase levels in the brain were not significantly altered (88% of controls) while the activity of brain ChE was greatly reduced (15% of controls). No signs delayed neuropathy were observed.

On file at DPR is a study in hens conducted by Beavers *et al*. (1990). This study was judged acceptable for filling the SB950 data requirement for delayed neurotoxicity testing. In this study, 16 adult atropinized hens were given a single initial oral dose (corn oil vehicle) of 250 mg/kg methyl parathion (95.8% pure) and a subsequent dosing at 215 mg/kg (LD_{50}) 21 days afterwards. No clinical signs of delayed neuropathy, ataxia, or histopathological findings were detected.

15. IMMUNOTOXICITY

Several studies from the open literature showed that methyl parathion has the potential to alter the immune system. However, further research is needed to clearly identify the health implications of some of these immunological changes.

Shtenberg and Dzhunusova (1968) reported a decreased agglutinin titer (1:33 to 1:75, compared to the 1:1200 in the controls) in rats vaccinated with NIISI polyvaccine either 2 weeks before or after, or simultaneous to, the administration of metaphos (the USSR common name for methyl parathion) in the

diet at 1.25 mg/kg/day. Samedov *et al*. (1979; as cited in USEPA, 1984) noted reductions in phagocytic activity of leukocytes, complement titer, serum lysozyme activity, and nucleic acid content of blood in rabbits that received 5 mg/kg/day methyl parathion in sunflower oil, 6 days/week for 4 months.

In a study by Street and Sharma (1975), male rabbits were fed diets containing methyl parathion at 0, 0.6, 2.6, 8.5, or 23 ppm (0, 0.036, 0.16, 0.52, and 1.5 mg/kg/day) for 8 weeks. The treatments did not result in any gross toxicity. At week 4, rabbits were challenged with sheep erythrocytes. The number of gamma-globulin producing plasma cells was significantly decreased ($p<0.05$) in popliteal lymph nodes at all dose levels. Germinal centers in the splenic white pulp were reduced to the same extent at 8.5 and 23 ppm. Total and differential leukocyte counts, hemolysin and hemagglutinin titers, serum gamma-globulin/transferrin ratio, tuberculin reactivity, food consumption, and body and organ weights were not affected. A dose-related increase in the degree of thymus cortex atrophy was observed. The scoring was statistically significant ($p<0.05$) at the 8.5 ppm (1.5 mg/kg/day).

Using cultured human lymphocytes and neutrophils, Park and Lee (1978) reported that methyl parathion at 10 FM showed approximately 25% inhibition in the response of neutrophils to chemotactic stimuli. The responses of lymphocytes to phytochemagglutinin stimulation were 79 and 89% of the control for the whole blood and isolated mononuclear cells. These levels were reported as not statistically significant ($p>0.025$).

In the 3-month rat study by Daly and Rinehart (1980a) (see: Section 7.2., *Oral Studies in Rats*), lymphoid depletion and necrosis of lymph nodes, spleen, and thymus were noted in rats that were fed diets containing 75 ppm methyl parathion and died within four weeks of dosing. Fan (1980) reported an increase in mortality associated with i.p. challenge of *S. typhimurium* in male Swiss-Webster mice that received 3.0 mg/kg/day methyl parathion in the diet for at least 2 weeks. Increased viable bacteria in the blood, decreased total gamma-globulin and IgG, and reduced response of splenic lymphocytes to mitogen stimulation were concomitantly observed. No effects were reported at the two lower doses (0.08 or 0.7 mg/kg/day).

Rodgers *et al*. (1986) studied the effects of methyl parathion on the cytotoxic T-lymphocyte (CTL) response in mice (C57B1/6) splenocytes. Following a 1-hour incubation with methyl parathion (with or without the pre-treatment of NADPH-fortified S-9 microsomal enzyme fraction), the splenocytes were sensitized to allogenic tumor target cells. The CTL response was subsequently determined based on the release of ^{51}Cr from radiolabeled target cells. Methyl parathion at 5-10 Fg/ml (calculated as 19-38 FM) decreased the ability of splenocytes to generate a CTL response. With the S-9 pre-incubation, the effect of methyl parathion was 20-fold less than without the S-9.

Institoris *et al*. (1992) investigated the humoral immune effects of methyl parathion in mice. Male mice were administered methyl parathion (60% pure) prior to the exposure to sheep erythrocytes (SRBC). The purity-adjusted doses of methyl parathion were: 5.3 mg/kg in a single dose given 0-3 days prior to SRBC exposure, and 0.53 or 0.27 mg/kg given for 4 weeks. The authors reported increases in the number of plaque-forming splenocytes (PFC) with no changes in serum antibody titers. The effect, however, did not appear to be dose-related. The brief reporting precluded further evaluation of this study.

The potential for immunotoxicity after long-term exposures was recently reported by Institoris *et al*. (1995) in a 3-generation study in Wistar rats. The mating and treatment schedule of this study was similar to the protocol of a 3-generation reproductive toxicity study, except for having one instead of two litters per generation. Methyl parathion at 0, 0.218, 0.291, or 0.436 mg/kg was administered via intubation, 5 days per week. The effects that were statistically significant included: decrease in WBC (white blood cells), RBC, hematocrit (most prominent in G_1 - parent generation; at all methyl parathion dose levels), increase in medial RBC cell volume (in G_1), slight increase in relative liver weight (in G_1, G_2, G_3; all three generations) and decrease in relative thymus weight (in G_3), increase in the nucleated cell contents in the femoral bone marrow (in G_2, G_3), and dose-dependent decreases in PFC with SRBC (27% reduction at 0.436 mg/kg in G_1). It is interesting to note that while a decrease of PFC was noted in this study in rats, comparable levels of methyl parathion appeared to cause an increase, instead of decrease, of PFC in mice (Institoris *et al*., 1992).

A comprehensive study on the immunotoxicity of methyl parathion was recently conducted by Crittenden *et al*. (1998) in female B6C3F1 mice. The areas of study included dose-response and time-course studies (ChE activities, hematology, thymus/spleen weight and cellularity, antibody-forming cultures, peritoneal macrophage nitrite production, and natural killer cell (NK) activity), cell-mediated and humoral immunity, and host resistance (melanoma cells and *Streptococcus agalactiae)*. Groups of 5-8 mice received 0, 1, 3, or 6 mg/kg/day methyl parathion (>99% pure) via gavage for 7, 14, 21, and 28 days. No effects on body weight or hematology were reported. Brain and plasma ChE inhibitions were noted at or above 3 and 6 mg/kg/day, respectively. Parameters showing immunotoxicity were: splenocytes antibody-forming cells reduction, increased NK activities, and increased nitrite production by peritoneal macrophages. The authors concluded that the overall data did not suggest substantial immunotoxic potential for methyl parathion.

16. HEMATOLOGICAL EFFECTS

Hematological effects have been noted in many of the studies presented in this document. The effects commonly reported were: decreases in RBC, hemoglobin, and hematocrit. In addition to decreases in RBC and differential leukocytic counts, Galal *et al*. (1977) also noted an increase in coagulation time in rats after 36 days of increasing exposure (see: Section 7.2., *Oral Studies in Rats*). The LOELs for these effects are summarized below:

1) 5.7 mg/kg/day (75 ppm in the diet) from the 3-month rat study by Daly and Rinehart (1980a) - Section 7., *Subchronic Toxicity*

2) 2.6-5.0 mg/kg/day (50 ppm in the diet) from the 2-year rat study by Bomhard *et al*. (1981) - Section 8., *Chronic Toxicity*

3) 0.19-0.28 mg/kg/day (5.0 ppm in the diet) and 2.0-3.2 mg/kg/day (50 ppm in the diet) from the 25-28 months rat study by Daly and Hogan (1983) - Section 8., *Chronic Toxicity*

4) 0.218, 0.291, and 0.436 mg/kg (5 days per week) from the 3-generation intubation study in rats by Institoris *et al.* (1995) - Section 15., *Immunotoxicity*

The mechanism for these hematological effects is not known. Parent-Massin and Thouvenot (1993) investigated the effects of 12 pesticides on the hematopoietic lineage by several pesticides known to cause changes in hematological indices. The study was conducted with hematopoietic progenitor cultures of colony-forming unit-granulocyte and macrophage (CFU-GM) from humans (obtained from arthroplasty surgeries) and rats. The effects were examined on day 7, 10 and 14 of incubation with test media. Methyl parathion at 0.2, 2, and 20 Fg/ml resulted in growth reduction of human CFU-GM colonies (>50 cell aggregates) and clusters (5-50 cells) on day 10 and 14. No effects were noted in cell cultures from rats. It is important to note that based on the 12 pesticides tested, progenitor cell cultures from humans appeared to be generally more sensitive than the cultures from rats.

17. ENDOCRINE DISRUPTION POTENTIAL

The potential impact of environmental chemicals and complex mixtures on the endocrine or hormonal system of human and wildlife has been a subject of great concern in recent years. Environmental chemicals such as organochlorines (e.g., dichlorodiphenytrichloroethane, DDT; polychlorinated biphenyls, PCBs), have been shown to interact with the endocrine system in humans and domestic and wildlife species. The outcome may be manifested in cancer, or a number of reproductive and developmental anomalies, such as altered growth and development, reproduction and behavior, and/or chemical homeostasis in an organism (Cooper and Kavlock, 1997; EDSTAC, 1998). An endocrine disruptor may interfere with the role of natural hormones in several ways. It may affect hormonal production and synthesis, directly bind to the hormone receptors, or interfere with the breakdown of hormones (USEPA, 1997d).

Initially, the concern was mainly for those chemicals that interfered with normal actions of estrogen and thereby altering the natural development of the reproductive tract and sexual differentiation of the brain

(e.g., precocious puberty, disrupted cycling). However, the broad context of endocrine action necessitates the extension of the scope to include non-sex-steroid-based functions, such as thyroid, and potentially any natural hormones. While serious effects could occur over all life stages, of particular concern was the exposure during the developing stage when the organism may be more vulnerable and the effects long lasting (USEPA, 1997d).

The complexity of the issues with respect to the many potential mechanisms and target organs cannot be overstated. Presently, data to address these effects are largely lacking. Clearly, a collective effort within the scientific community is needed to address the many basic issues such as testing programs and endpoints, before a defined approach can be developed to assess the risk of endocrine disruption. In 1996, both the Food Quality Protection Act (FQPA) and the amendments to the Safe Drinking Water Act required USEPA to develop screening programs to determine the endocrine disruption potentials of environmental chemicals, including pesticides. Under FQPA, the screening and testing process for pesticides is expected to be implemented by August 1999.

Two of the many activities initiated by USEPA for addressing the concerns of endocrine disruption were the documentation of the state of the science by the Risk Assessment Forum, and the chartering of the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC). An overview on the effects assessment and analysis of endocrine disruption was published in 1997 (USEPA, 1997d). The EDSTAC final report was published in 1998 (EDSATC, 1998). The 1997 report under the auspices of Risk Assessment Forum served as a basis for USEPA Science Policy Council position. In light of the lack of sufficient information as well as consensus in the scientific community, USEPA's interim science policy stated that "*the Agency does not consider endocrine disruption to be an adverse endpoint per se, but rather to be a mode or mechanism of action potentially leading to other outcomes*." (USEPA, 1997d). The report further pointed to the need for research and testing programs to enable future risk reduction, especially to the young and the vulnerable.

Regarding developing a strategy for screening and testing substances for their potential to disrupt the endocrine system, the 1998 EDSTAC report made recommendations for priority setting as well as tests for endocrine disrupting chemicals. The scope for endocrine disruption screening and testing was defined to include effects in humans and wildlife, with a primary focus to the estrogen, androgen, and thyroid hormonal (EAT) systems, and the evaluation of common mixtures of contaminants. A tier approach prioritization scheme was recommended to be based on the EAT hormone systems, data availability, and evidence. All pesticides would undergo "High Throughput Pre-Screening" (HTPS) with Tier 1 screening assays expected to provide information on EAT receptor bindings, as well as generate data for prioritization, Quantitative Structure Activity Relationship (QSAR) models, and determining subsequent Tier 2 tests. A pilot program would be used to evaluate the feasibility and validation of this approach. In general, information on both the effects and exposures would be considered in the priority setting for the Tier 1 screening. For pesticides, common mixtures with fertilizer would be considered and the prioritization would follow the schedule for tolerance

reassessment under FQPA. In recommending batteries of screens and tests, EDSTAC emphasized the importance of validation and standardization. Recommended Tier 1 screening assays consisted of both *in vitro* and *in vivo* tests. The *in vitro* assays included estrogen and androgen receptor binding and reporter gene assays and steroidogenesis assay with minced testis. The *in vivo* assays included 3-day uterotrophic assay, 20-day assay with pubertal females including thyroid endpoints, 5-7-day Hershberger assay in rodents, and frog metamorphosis and fish gonadal recrudescence assays. Data from all of these assays would be necessary for an accurate decision about the chemicals. Alternative assays were also recommended. The Tier 2 tests included 2-generation mammalian reproductive toxicity study, and tests on avian reproduction, fish and mysid life cycles, and amphibian development and reproduction. An additional project would be performed to evaluate the adequacy of the conventional toxicology studies for assessing the endocrine active substances.

Information pertinent for the evaluation of endocrine disruption potential of methyl parathion is limited. Data specific to the reproductive and developmental toxicities of methyl parathion were presented in Section 11. and 12. Although the reproductive toxicity database (see: Section 11.) indicated that some OPs may affect the menstrual cycle and cause early menopause in humans, no data on human reproductive effects specific to methyl parathion are available. Decreased pup survival was the consistent reproductive toxicity endpoint in laboratory animals. Ovarian effects in rats, sperm abnormalities in mice, and testicular and reproductive effects in avian species were also reported. The developmental toxicity database in rats, rabbits, mice, and avian species (see: Section 12.) showed lower fetal body weight, increased resorption, reduced pup survival, and abnormalities and variations of ossification. There was also some indication of neurobehavioral effects in rats and a report of cleft palate and increased death in mice at a much higher dose. Injection of methyl parathion into the air space of chicken eggs resulted in cervical lordosis and scoliosis, cervical muscle atrophy, lower body weight, and retarded growth. Unfortunately, endocrine activities had not been a consistent focus for reproductive and developmental toxicity studies. Some of the endpoints pertinent to the EAT systems were added to the current guidelines for the 2-generation reproductive toxicity study (Health Effects Test Guidelines, OPPTS 870.3800) published in 1998 (USEPA, 1998d). Specifically, the new guidelines call for more comprehensive observations (e.g., estrus cycle, sperm enumeration, motility and morphology) and histopathology for the parental males and females.

Among all the above data, the reports on ovarian toxicity by Dhondup and Laliwal (1997) and Asmathbanu and Kaliwal (1997) in rats and the study on male reproductive hormone profile among workers by Padungtod *et al*. (1998) appeared to provide some indication of endocrine disruption potential. These studies were presented in Section 11., *REPRODUCTIVE TOXICITY*. Dhondup and Laliwal (1997) and Asmathbanu and Kaliwal (1997) reported lengthened estrous cycles, and reduced compensatory ovarian hypertorphy, healthy follicles, and relative uterus weight in hemicastrated rats treated with 2.5 to 5.0 mg/kg/day methyl parathion for up to 15 day. Padungtod *et al*. (1998) reported a significant correlation between serum luteinizing hormone and occupational exposure of 34 male workers in a factory in China manufacturing methyl and ethyl parathion and methidathion.

Using two bioassay systems to determine the direct interaction between estrogen receptor and estrogenic compounds, Petit *et al*. (1997) tested the estrogenic activity of 49 pesticides, environmental chemicals, and phytoestrogens. The first test used recombinant yeast system which contained a reporter gene with two estrogen-responsive elements, the induction of which is dependent on the rainbow trout estrogen receptor (rtER) and estrogens. The second test for expressing vitellogenin gene in rainbow trout hepatocyte aggregate culture was subsequently used as a complementary assay. Methyl parathion showed weak estrogenic activity in the yeast system but was highly estrogenic in the hepatocyte aggregate cultures. The authors speculated that metabolic transformation of methyl parathion by the hepatocytes may account for the high estrogenic activity.

In conclusion, the existing data indicated that methyl parathion may possess endocrine disruption potential. Although reproductive and teratogenic effects of methyl parathion had been reported in laboratory animals, the underlying mechanisms for these effects were not known. Neither were endocrine activities a part of these study protocols. As is generally recognized, testing guidelines and criteria for hazard identification are needed for a clear evaluation of the endocrine disruption potential.

18. RISK ANALYSIS

Risk analysis consists of four components: Hazard Identification, Dose-Response Assessment, Exposure Assessment, and Risk Characterization. In Hazard Identification, the intrinsic toxicity or hazard of a risk agent is identified. Dose-Response Assessment delineates the relationship between the dose and the severity of the identified health hazards. It establishes the threshold for non-oncogenic effects and, when applicable, the slope for oncogenic effects. The potential human exposure to the risk agent is estimated in the Exposure Assessment. Based on the data from the aforementioned three components, the potential risk of human exposures is then characterized or estimated in the Risk Characterization. Inherent in the risk analysis is the use of assumptions and defaults where data are inadequate or unavailable. The associated uncertainties are subsequently highlighted in the Risk Appraisal section.

18.1. RISK ANALYSIS - Hazard Identification and Dose-Response Assessment

Methyl parathion is a Category I pesticide based on its acute toxicity. Although methyl parathion is genotoxic in laboratory studies and there is some limited evidence of oncogenicity in rodent bioassays (See: Section 18.1.4), the weight of evidence is insufficient for a quantitative assessment of oncogenic risk The characterization of the risk of methyl parathion in this document is based on non-oncogenic effects.

The International Agency for Research on Cancer (IARC) placed methyl parathion in Category 3 which denotes chemicals not classifiable as to their oncogenicity in humans (IARC, 1987). Based on the lack of evidence for oncogenicity in the two chronic/oncogenicity studies in rats (Bomhard *et al*., 1981; Daly and Hogan, 1983) and the one study in mice (Eiben, 1991), USEPA (1998c, 1999) determined that methyl parathion should be classified in "Group E" (Evidence of Non-carcinogenicity of Humans) regarding human carcinogenicity potential as defined in the USEPA 1986 carcinogen risk assessment guidelines (USEPA, 1986b). Following the USEPA 1996 proposed carcinogen risk assessment guidelines (USEPA, 1996c), methyl parathion would be classified in the "Not Likely" group pertaining to its carcinogenic potential in humans via relevant routes of exposure (USEPA, 1998c, 1999). In this document (see: Section 18.1.4), the lack of clear oncogenicity evidence in the bioassays was discussed in the context of genotoxicity and the alkylating potentials of methyl parathion, and the effect of *ad libitum* feeding on the power for detection.

In risk assessment, two terms have commonly been used in delineating the threshold dose for non-oncogenic effects. The NOEL is the experimentally determined highest dose at which no effects were observed. The term No-Observed-Adverse-Effect Level (NOAEL) is sometimes used to emphasize the adversity of the endpoints that formed the basis of the NOEL. As the definition of adversity of effects is sometimes subjective, no distinction between the NOEL and NOAEL is made in this document for characterizing the risk. One key issue regarding the adversity of endpoints, especially for

the neurotoxicity of organophosphate chemicals has been the inhibition of ChE activities in the plasma and the RBC. In this document, the NOELs are presented in the context of the LOEL which is the lowest dose in the experiment at which hazard or toxicity was observed. This included both the effects on ChE inhibition (plasma, RBC, brain) and other overt effects. In a toxicity study, the LOEL is the next higher dose above the NOEL.

For characterizing the risk of inhalation exposures of contaminants in the air, data from inhalation toxicity studies are preferable. However, the only studies available for the NOEL and LOEL determinations were those in which methyl parathion was administered through the oral route. The lack of studies for delineating an inhalation NOEL necessitates the extrapolation of data from the oral route. Important to route-to-route extrapolation are considerations for both pharmacokinetics (e.g., route-specific absorption factors) and toxicological endpoints. Data presented in Section 3., *PHARMACOKINETICS*, indicated that the extent of methyl parathion absorption is comparable between oral and inhalation routes and can practically be considered as 100%. Since the toxicological effects identified in the available studies are systemic rather than localized (i.e., at the site of contact), the oral NOELs and LOELs can therefore reasonably be assumed to be the thresholds for inhalation exposures. No data are available for a cross-route comparison in humans. Therefore, it is further assumed that the information in animals would similarly apply to humans.

18.1.1. Acute NOEL

A list of NOELs and LOELs is given in Table 22. It includes the thresholds established from all pertinent studies described in Section 6., *ACUTE TOXICITY*, and under any other toxicity categories pertinent to acute exposures, e.g., the threshold for developmental effects that can potentially occur after a single exposure *in utero*. Thresholds based on both ChE inhibitions and overt toxicities (e.g., clinical signs, developmental endpoints) are presented.

ChE Inhibition

A series of studies by Rider *et al.* (1969; 1970 and 1971) provided NOELs determined in humans (Table 22). The focus of these studies was on the effects of plasma and RBC ChE activities. In the earlier study (Rider *et al.*, 1969), test subjects were given increasing doses of methyl parathion from 1 mg/day to 19 mg/day in 33 days. Test subjects in the subsequent studies (Rider *et al*., 1970, 1971) received fixed doses for 30 days. Greater detail of investigative protocol was reported in the earlier study while the results of the subsequent studies were published only as abstracts. Although the studies differed in the dosing schedule, it is reasonable to assume that the investigative protocols were similar for all the studies by the same authors. The NOELs from these studies were comparable. The 30-day study (Rider *et al.*, 1970, 1971) had a slightly higher NOEL of 0.31 mg/kg/day. It was based on ChE inhibitions of 23% in the plasma and 55% in the RBC reported at the LOEL of 0.34 mg/kg/day (Table 4).

Table 22. Acute No-Observed-Effect Levels (NOELs) and Lowest-Observed-Effect Levels (LOELs) of methyl parathion[a].

Study/ Species	ChE Inhibition ChE	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Overt Toxicity Effects at the LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)	References
				Studies in Humans			
1 day[b]	pl, rbc	0.27	-	no effects observed	0.27	-	Rider *et al*., 1969
5-day	pl, rbc	0.057	-	no effects observed	0.057	-	Rodnitzky *et al*.,1978
30-day	pl, rbc	0.31	0.34	-	-	-	Rider *et al*., 1970, 1971
				Studies in Rats			
1-day		-	-	salivation, "shivering", lacrimation, exophthalmos, hyperreflexia, respiratory distress	-	5.3	Galal *et al*., 1977
1-day		-	-	nasal/oral discharge, wet rales, 9general activities	-	1.0	Auletta, 1984a
6 weeks		-	-	mortality (1st week only)	-	0.22	Schulz *et al*., 1990
1-day (pup, 15 days old)	br	-	1.0	"shivering", salivation, muscular fasciculation	-	1.0	Kumar and Desiraju,1992
1-day	pl, rbc, br	0.025	7.5	focal demyelination of peripheral nerves	0.025	7.5	Minnema, 1994a

(continued)

Table 22. Acute No-Observed-Effect Levels (NOELs) and Lowest-Observed-Effect Levels (LOELs) of methyl parathion[a]. (continued)

Study/ Species	ChE Inhibition ChE	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Overt Toxicity Effects at the LOEL	NOEL (mg/kg/day)	LOEL (mg/kg/day)	References
				Developmental Toxicity Studies			
Rat				dam/fetal: body wt↓(fetus: 5%; dam: 43%)	0.3	1.0	Machemer, 1977
Rat		-	-	pup: day 15 survival; neurobehavior (reflex, open-field, maze)	-	1.0	Crowder *et al.*, 1980
Rat	br (dam/pup)	-	1.0	dam: muscle fasciculations, tremors (start day 3-4)	1.0	1.5	Gupta *et al.*, 1985
Rat		-	3.0	dam: death (start day 7), wt↓, cholinergic signs (start day 8), fetal : wt↓, delayed ossification	1.0	3.0	Becker *et al.*, 1987*
Rabbit	rbc (dam)	-	0.3	fetal: thickened areas of ossification	3.0	9.0	Hoberman, 1991*

a/ The route of exposure is oral for all studies. ChE: cholinesterase inhibited at the LOEL; br: brain; pl: plasma; rbc: red blood cell.

b/ Male subjects were exposed to daily increasing doses for a total test period of 33 days.

* study or studies that fulfilled the SB950 data requirement for the specific type of testing.

For the lack of any other better quality studies to determine an acute NOEL in humans, the subchronic NOEL of 0.31 mg/kg/day was used to assess the risk associated with acute exposures. The NOEL for a shorter term of exposure for the same endpoints of ChE inhibition might be higher. This NOEL was also used by the U.S. EPA in setting a 10-day drinking water Health Advisory (USEPA, 1988). No clinical signs of toxicity were reported at doses as high as 30 mg/day (0.43 mg/kg/day), the highest dose tested. However, these studies were not designed for detecting more subtle neurological effects.

Studies in laboratory animals provided the thresholds for endpoints not examined in the above human studies. To facilitate the determination of critical NOELs for characterizing the risk of acute exposures, essential information from those studies listed in Table 22 representing the lowest NOELs or LOELs are briefly summarized below.

The lowest NOEL for plasma, RBC, and brain ChE inhibition was 0.025 mg/kg/day in rats (Minnema, 1994a), based on substantial inhibitions of plasma, RBC, and brain ChE at a LOEL of 7.5 mg/kg/day. This single dosing study by Minnema (1994a) was presented in Section 13.1., under *NEUROTOXICITY*. With the marked difference between the NOEL and LOEL (i.e., 300-fold), a question could be raised regarding the possibility that the NOEL could be higher had the study design reduced the dose interval within this region. On the other hand, it should be noted that there was a 22% plasma ChE inhibition at this NOEL, although not statistically significant.

The lowest LOEL for RBC ChE inhibition was 0.3 mg/kg/day in rabbits from the study by Hoberman (1991), as presented in Section 12.2. of the *DEVELOPMENTAL TOXICITY*. The lowest LOEL for brain ChE inhibition was 1 mg/kg/day in rats (Kumar and Desiraju, 1992; Gupta *et al*., 1985 in Table 22). The study by Kumar and Desiraju (1992) was presented in Section 13.1., under *NEUROTOXICITY*. Rat pups (15 days old) that received a single oral dosing of 1 mg/kg methyl parathion orally showed 47% ChE inhibition in the brain stem region. The study by Gupta *et al*. (1985) was presented in Section 12.1. of the *DEVELOPMENTAL TOXICITY*. As high as 36% ChE inhibition was reported in 3 of the 4 brain regions (frontal cortex, brain stem, striatum) of the pups from dams that orally received 1 mg/kg/day methyl parathion during gestation day 6 through day 20.

When the LOEL is the lowest dose tested in a study, a default factor of 10 is often used to estimate the NOEL. Applying the default factor to the aforementioned LOELs, the estimated NOELs would be 0.1 mg/kg/day for brain ChE inhibition in rats and 0.03 mg/kg/day for RBC ChE inhibition in rabbits. However, without taking into account the shape of the dose response curve and the level of response at the LOEL, this default approach introduced substantial uncertainties. A NOEL that can be determined directly from a quality study would afford a greater certainty.

Overt Toxicity

The lowest LOEL reported for the overt effects was 1.0 mg/kg/day (Auletta, 1984a; Kumar and Desiraju, 1992; Crowder *et al*., 1980), with the exception of the 0.22 mg/kg/day reported by Schulz *et al.* (1990). The study by Auletta (1984a) was described in Section 6.3.2. Cholinergic signs occurred after a single exposure of 1 mg/kg/day. The study by Kumar and Desiraju (1992) was presented in Section 13.1., under *NEUROTOXICITY*). Rat pups (15 days old) that received a single oral dosing of 1 mg/kg methyl parathion showed cholinergic signs of "shivering", salivation, and muscular fasciculation. The study by Crowder *et al.* (1980) was described in Section 12.1. The effects noted in the pups of dams that received methyl parathion orally during gestation day 7 through day 15 included reduced postpartum day 15 survival and neurobehavioral effects. There were substantial uncertainties associated with the increase in mortality reported in the study by Schulz *et al.* (1990) (see: Section 6.3.2.). Of the 20 Wistar rats per group, 3 and 4 rats reportedly died at the respective dose levels of 0.22 and 0.44 mg/kg/day, all during the first week of treatment. However, during the same period, death also occurred in one out of the 20 control rats (both the controls that were treated with tap water and the controls that were similarly handled but not intubated). No cause of death was reported and no additional mortality occurred subsequently throughout the remaining 5 weeks of treatment. It is also important to note that although mortality is an expected acute endpoint, none of the studies from the sizable toxicological database reported death occurring at such a low dose level. The lowest acute NOEL for overt effects was 0.025 mg/kg, based on a clear increase in nerve demyelination at the LOEL of 7.5 mg/kg (Minnema, 1994a). The possibilities of a higher NOEL due to the 300-fold difference between the NOEL and the LOEL should be viewed in the context of the apparent slight increase of demyelination in the lumbar root fibers at the NOEL.

The NOEL of 0.025 mg/kg/day from the acute study was originally used by USEPA (1998c) in the draft Reregistration Eligibility Decision document (RED) to assess the acute and short-term risk of methyl parathion exposures. Considering the 300-fold difference between the NOEL and LOEL, USEPA recently determined that the NOEL of 0.1 mg/kg/day from the 1-year chronic toxicity study in rats by Daly (1991) is most appropriate for use as the NOEL for acute, short-, and intermediate-term toxicity (USEPA, 1999). It should be noted that in this DPR assessment (Section 8.1), the NOEL from the 1-year study by Daly (1991) was determined to be at 0.02 mg/kg/day.

When using a NOEL determined in animals to characterize the risk of human exposure to toxicants, it has generally been assumed that humans can be 10 times more sensitive than animals. Therefore, the NOEL for humans would be 0.0025 mg/kg/day when extrapolated from the NOEL of 0.025 mg/kg/day in rats based on ChE inhibitions (plasma, RBC, brain) and nerve demyelination. This level is 23-fold lower than the NOEL of 0.057 mg/kg/day determined in two human adult males from the study by Rodnitzky *et al.* (1978) described in Section 7.1. In addition to the difference in species, several

key elements might have contributed to the ifference in NOELs. Some of these factors included the small sample size of two test subjects in the human study by Rodnitzky *et al*. (1978) and the endpoint of brain ChE and histopathological examinations of nerves which cannot be monitored in humans. Age might be another possible factor. The animal studies included rat pups on post-natal day 15 (Kumar and Desiraju, 1992), *in utero* exposure scenario (Crowder *et al.*, 1980), and relatively young rats of 7-8 weeks old (Minnema, 1994a).

Conclusions

Although the use of a NOEL determined in humans avoids the uncertainties associated with the inter-species extrapolation, these studies were either focusing mainly on the effects of ChE inhibition or did not have sufficient sample size. Therefore, for ChE inhibition, both the NOEL of 0.31 mg/kg/day established from the studies in humans and the NOEL of 0.025 mg/kg/day based on studies in laboratory animals would be used to characterize the risk of acute exposures. The NOEL for nerve demyelination in rats was also 0.025 mg/kg/day. The uncertainties associated with the NOELs as presented above are described in Section 18.4, RISK ANALYSIS - Risk Appraisal.

18.1.2. Subchronic NOEL

Lists of NOELs and LOELs are given in Table 23 for the effects of ChE inhibitions and in Table 24 for overt effects. These lists included pertinent studies described in Section 7., *SUBCHRONIC TOXICITY* and under any other toxicity categories pertinent to subchronic exposures, e.g., the threshold for reproductive toxicities. In addition, data presented in Table 5 on ChE inhibitions from the chronic toxicity studies were also included for the NOEL determination when the ChE activities were measured during a subchronic time interval (i.e., up to 3-6 months).

ChE Inhibition

A human NOEL of 0.31 mg/kg/day was determined based on the inhibition of plasma and RBC ChE at 0.34 mg/kg/day. However, uncertainties existed with only 5 adult subjects in the test groups and the brief reporting (only as abstracts for platform presentations). Also, the lack of data in brain ChE measurement would dictate that a critical NOEL for risk assessment should also take into account the data from animal studies.

The lowest NOEL for plasma ChE inhibition was 0.1 mg/kg/day in Sprague-Dawley rats after receiving 2.5 ppm in the diet for 1 month. This NOEL was established from the study by Daly (1991) as presented in Section 8.1., under *CHRONIC TOXICITY,* and in Table 5. At the LOEL

Table 23. Subchronic No-Observed-Effect Levels (NOELs) and Lowest-Observed-Effect Levels (LOELs) of methyl parathion based on cholinesterase inhibition [a].

Species/ Duration	ChE at the LOEL[b] (% inhibition)	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Reference
Humans; 30 days	pl (23%), rbc (55%)	0.31	0.34	Rider *et al*., 1970,1971
Rats; Sprague-Dawley				
1-3 months	pl (39%), rbc (43%), br(32%)	0.2[c]	1.9	Daly & Rinehart, 1980a
1 month	pl (33%), rbc (13%)	0.1	0.48	Daly, 1991
13 weeks	rbc (28%)	0.029	0.29	Minnema, 1994b
Rats; Wistar				
10 days	pl (76%), br (57%)	-	1.3	Yamamoto *et al*., 1982
15days (2 days old)	br (no data in report)	-	0.1	Kumar & Desiraju, 1992
150 days (2 days old)	br (5 regions, $24%)	-	0.2	Kumar & Desiraju, 1992
2-13 weeks	rbc (4-20%)	-	0.09	Bomhard *et al*., 1981
Mice; B6C3F1				
65-66 days	br (20-30%)	3.82	14.91	Eiben, 1988a,b
	pl (10%); rbc (8%)	-	0.93	
Dogs				
3 months	br (56-64%)	1.0	3	Underwood &
	pl (28%), rbc (37%)	0.3	1.0	Tegeris,1978
13 weeks	br (50-53%)	0.3	3	Daly, 1989
	pl (19%)	-	0.03	
1-6 months	pl (31%)	-	0.3	Hatch, 1998
	rbc (37%)	0.3	1.0	

a/ The route of exposure is oral for all studies.

b/ ChE: cholinesterase activities, given in the highest group percentage of inhibition as compared to the controls. pl: plasma; rbc: red blood cell; br: brain.

c/ At the NOEL, rbc ChE was inhibited by 30% at 1 month, but no significant inhibition was shown at 3 months.

Table 24. Subchronic No-Observed-Effect Levels (NOELs) and Lowest-Observed-Effect Levels (LOELs) of methyl parathion based on overt toxicities[a].

Species/ Study	Effects at the LOEL	NOEL (mg/kg/day)	LOEL	Ref
Rats; Sprague-Dawley				
Repro study	pup survival (~5%9); dam body weight gain reduction	0.4	2.3	Daly & Hogan, 1982
13 weeks	alopecia and skin sore	-	0.029	Minnema, 1994b
Rats; Wistar				
Rat; Wistar	8urination, latency to leave center and rearing; 9grooming, 9defecation	-	0.22-0.44	Schulz *et al.*,1990
Repro study	pup survival (814%- 913%)	0.14	0.71	Loser & Eiben,1982
Rats; Fischer F344				
7 weeks	death in 1 of 5 females	-	0.5	NCI, 1979
Mice, B6C3F1				
7 weeks	death of 1 in 5 males at the LOEL; no death in any higher dose groups	-	2	NCI, 1979
Dogs				
14 days	vomiting in one of 2 dogs	-	2.5	Underwood & Tegeris, 1977
13 weeks	reduced intraocular pressure	0.03	0.3	Daly, 1989

a/ The route of exposure is oral for all studies. Studies listed in Table 23 were not repeated unless the NOELs were at or below those listed in Table 23 for brain ChE inhibitions. Repro: reproductive toxicity study.

of 0.48 mg/kg/day (12.5 ppm in the diet), the 25% inhibition in the male rats after 1 month of treatment was statistically significant, whereas the 33% inhibition in the females was greater but did not show statistical significance. On the other hand, at a lower dose of 0.03 mg/kg/day, the study by Daly (1989) reported a 19% inhibition of plasma ChE in dogs after 13 weeks of dosing (see Section 7.4., under *SUBCHRONIC TOXICITY* and Table 4). Since a NOEL cannot be determined from this study, using a default factor of 10, the estimated NOEL for plasma ChE inhibition was 0.003 mg/kg/day.

The lowest NOEL for RBC ChE inhibition was 0.029 mg/kg/day in Sprague-Dawley rats based on the study by Minnema (1994b). However, a LOEL of 0.09 mg/kg/day was identified in Wistar rats based on the study by Bomhard *et al*. (1981) as presented in Section 8.1., under *CHRONIC TOXICITY*, and in Table 5. At this LOEL, the RBC ChE was statistically significantly inhibited by 4-20% after receiving 2 ppm in the diet for 2 - 13 weeks. Since a NOEL cannot be determined from this study, using a default factor of 10, the estimated NOEL for RBC ChE inhibition was 0.01 mg/kg/day. This NOEL was 3-fold lower than the NOEL established in Sprague-Dawley rats but with a greater uncertainty due to the application of a default factor.

The lowest NOEL for brain ChE inhibition was 0.2 mg/kg/day in Sprague-Dawley rats based on the 3-month study by Daly and Rinehart (1980a). The study was presented in Section 7.2., under *SUBCHRONIC TOXICITY*, and in Table 4. The brain ChE was inhibited by 5-32% at the LOEL of 1.9 mg/kg/day (25 ppm in the diet). However, at 0.2 mg/kg/day, Kumar and Desiraju (1992) reported at least 24% ChE inhibition in 5 regions of the brain in young Wistar rats after 150 days of dosing, starting on post-natal day 2. This was not surprising in light of the understanding that young rats appeared to be more sensitive (see: Section 6.2.3., *Age-related sensitivity*) to the acute toxicity of methyl parathion and that the two strains of rats may have different levels of sensitivity. The same authors also reported brain ChE inhibition in rats of the same age that were similarly treated with 0.1 mg/kg/day for only 15 days. However, there was a greater uncertainty in the LOEL of 0.1 mg/kg/day because the actual data for the ChE inhibition were not reported. Since a NOEL cannot be determined from this study, using a default factor of 10 and based on the LOEL of 0.2 mg/kg/day, the estimated NOEL for brain ChE inhibition was 0.02 mg/kg/day.

Overt Toxicity

The lowest subchronic NOEL based on overt toxicities was 0.03 mg/kg/day in dogs, based on a reduction of intraocular pressure after 13 weeks of exposure at 0.3 mg/kg/day (Table 24). The study by Daly (1989) was presented in Section 7.4., under *SUBCHRONIC TOXICITY*. However, in a recent 1-year study in dogs by Hatch (1998) (see: Section 8.3., under *CHRONIC TOXICITY*) no treatment-related ophthalmological changes (intraocular pressure, electroretinogram) were reported in dogs that received 0.3 to 4.0 mg/kg/day for up to one year.

The next set of higher NOELs was identified in two reproductive toxicity studies presented in Section 11.2. under *REPRODUCTIVE TOXICITY*. The NOEL of 0.14 mg/kg/day (2 ppm in the diet) in

Wistar rats was based on as much as 13% reduction of pup survival at the LOEL of 0.71 mg/kg/day (10 ppm in the diet) (Loser and Eiben, 1982). The NOEL of 0.4 mg/kg/day (5 ppm in the diet) in Sprague-Dawley rats was based on an approximately 5% reduction in pup survival and a reduction in maternal body weight gain at the LOEL of 2.3 mg/kg/day (25 ppm in the diet) (Daly and Hogan, 1982). Since the effect has consistently been noted in the reproductive toxicity studies, DPR's data review determined that considering these studies collectively, a NOEL for pup survival was 0.4 mg/kg/day. However, it should be noted that applying the approach for a collective NOEL to these two studies using two different strains of rats would assume that there was no sensitivity differences between the Wistar and Sprague-Dawley rats specific to this endpoint.

Two low LOELs for overt toxicities were presented in Table 24. A LOEL of 0.22-0.44 mg/kg/day was based on the neurobehavioral effects reported by Schulz *et al*. (1990) in Wistar rats after 6 weeks of oral dosing. Another LOEL of 0.029 mg/kg/day (0.5 ppm in the diet) was based on alopecia and skin sores which occurred in rats within 13 weeks of dosing (Minnema, 1994b). Additional support for the LOEL of 0.22-0.44 mg/kg/day were effects noted within the same dose range: 1) reduced pup survival at 0.71 mg/kg/day in the above 3-generation reproductive study in Wistar rats, with the NOEL of 0.14 mg/kg/day (Loser and Eiben, 1982), 2) a statistically significant increase in the degree of thymus atrophy in rabbits 8 weeks after receiving 0.52 mg/kg/day methyl parathion in the diet (Street and Sharma, 1975), and 3) in addition to a decreased relative thymus weight, a 3-generation rat study showing hematological changes (decreased RBC, WBC, hematocrit) at 0.218 mg/kg/day (the lowest dose tested) (Institoris *et al*., 1995). Applying the default factor of 10 and based on the LOEL of 0.22 mg/kg/day for the neurobehavioral effects (Schulz *at al*., 1990), the estimated NOEL was 0.02 mg/kg/day. This was in the same range as the estimated subchronic NOEL based on brain ChE inhibition.

A NOEL was not estimated from the LOEL of 0.029 mg/kg/day based on the slight increase of alopecia and skin sores. There were some uncertainties associated with the LOEL because the incidence of these effects was similar at the next higher dose of 0.29 mg/kg/day. Nevertheless, these observations would support the above estimated NOEL of 0.02 mg/kg/day.

Conclusions

The subchronic NOELs for ChE inhibitions were: 0.003 mg/kg/day for plasma ChE inhibition estimated in dogs; 0.029 mg/kg/day for RBC ChE inhibition established in Sprague-Dawley rats; and 0.02 mg/kg/day estimated for brain ChE inhibition. The NOEL for neurobehavioral effects was 0.02 mg/kg/day in Wistar rats. It should be noted that a NOEL of 0.02 mg/kg/day was also used by USEPA (1998c) in the Reregistration Eligibility Decision Document (RED) to assess the intermediate-term (subchronic) risk of methyl parathion exposures. This was based on the demyelination of nerves in the one-year chronic toxicity study in rats by Daly (1991) (see: the following Section 18.1.3.)

The above NOELs were lower than the NOEL determined in humans for plasma and RBC ChE inhibition by Rider *et al*. (1970, 1971). Although using a NOEL determined in humans will reduce the uncertainties for extrapolating data from animals to humans, the NOEL of 0.31 mg/kg/day did not account for the potentially more sensitive endpoints as tested in the animal studies. Thus, together with the NOEL of 0.31 mg/kg/day determined in humans, the NOELs for ChE inhibitions and neurobehavioral effects estimated from laboratory animals would be used as the critical NOELs in this document to characterize the risk of seasonal exposures.

In 1988, USEPA used the NOEL of 0.3 mg/kg/day based on plasma and RBC ChE inhibitions from the 3-month dog study by Underwood and Tegeris (1978) to set a long-term drinking water Health Advisory (USEPA, 1988).

18.1.3. Chronic NOEL

A list of NOELs and LOELs is given in Table 25 for pertinent studies described in Section 8., *CHRONIC TOXICITY*. This list included endpoints of ChE inhibition and other overt toxicities.

ChE Inhibition

Three NOELs for plasma ChE inhibition were available: 0.19 mg/kg/day in Sprague-Dawley rats (Daly and Hogan, 1983), 0.1 mg/kg/day in Sprague-Dawley rats (Daly, 1991), and 0.3 mg/kg/day in dogs (Hatch, 1998). A collective NOEL of 0.19 mg/kg/day could be determined from the two rat studies, since they were conducted in the same strain of rats and by the same investigator. Although conducted 8 years apart, the two studies showed a similar level of plasma ChE inhibition (63% and 67%) at 50 ppm, the common high dose level used in both studies.
The NOEL for RBC ChE inhibition was 0.1 mg/kg/day in Sprague-Dawley rats (Daly, 1991). The two LOELs for RBC ChE inhibition were: 0.09 mg/kg/day in Wistar rats (Bomhard *et al*., 1981), and 0.3 mg/kg/day in dogs (Hatch, 1998). Since a NOEL cannot be determined from the lower LOEL by Bomhard *et al*. (1981), using the default factor of 10 would result in an estimated NOEL of 0.01 mg/kg/day. It should be noted that in the study by Daly and Hogan (1983) in Sprague-Dawley rats, a 4-11% RBC ChE inhibition was noted at the NOEL of 0.19 mg/kg/day throughout the study, although the inhibition was not statistically significant at all time points. Based on this consideration, the NOEL for RBC ChE would have been 0.02 mg/kg/day, only twice higher than the estimated NOEL of 0.01 mg/kg/day in Wistar rats.

The lowest NOELs for brain ChE inhibition was 0.09 mg/kg/day in Wistar rats (Bomhard *et al*., 1981). The lowest LOEL was 0.2 mg/kg/day in B6C3F1 mice (Eiben, 1991). As low as 19% brain ChE inhibition was reported at this LOEL, although it did not showed statistical significance. Applying the default factor of 10, the estimated NOEL was 0.02 mg/kg/day in mice. However, it should be noted that the overall database did not show a general pattern of higher sensitivity in mice.

Table 25. Chronic No-Observed-Effect Levels (NOELs) and Lowest-Observed-Effect Levels (LOELs) of methyl parathion[a].

Species/ Study	Toxicity endpoints at the LOEL	NOEL (mg/kg/day)	LOEL	Reference
Rats; Sprague-Dawley				
25-28 months	br ChE (72%♀), pl ChE (79%♀)	0.19[b]	2.0	Daly & Hogan,1983
	abnormal gait, hematological alterations	0.02	0.19	
12 months	ChE inhibition (pl: 33%; rbc: 19%; br 25%)	0.1	0.48	Daly, 1991
	proximal sciatic, tibial/peroneal nerve myelin degeneration	0.02	0.1	
Rats; Wistar				
2 years	rbc ChE (17% ♀; 1 yr)	-	0.09	Bomhard *et al*.,1981
	br ChE ((22% ♀; 2 yr)	0.09	0.46	
3-generation	change in EEG index in somatosensory, visual, and auditory areas of cortex. Greater effects in 2nd and 3rd generations	-	0.22	Nagymajtenyi *et al*., 1995
Mice; B6C3F1				
104 weeks	br ChE (2 yr; 19%♀, statistically insignificant)	-	0.2	Eiben, 1991
	br ChE (1 yr; 37%♀, statistically significant)	0.2	1.6	
	increase in body weight and relative organ weight; poor general condition, tremors, paralysis	1.6	9.2	
Dogs				
1 year	br ChE (22%♀)	0.1	0.3	Ahmed & Sagartz, 1981
1 yr	rbc ChE (21%♀); pl ChE (31%♀)	-	0.3	Hatch, 1998
	br ChE (25%♀)	0.3	1	
	diarrhea, thinness, relative organ weight (♂adrenal, ♀spleen), ♀thymus lymphoid cells, tremors	1	3.5-4	

a/ The route of exposure is oral for all studies. The percentage of ChE inhibition represented the lowest group mean comparison to the controls. Studies by Daly & Hogan, 1983 and Hatch, 1998 were accepted for filling the data requirement for chronic toxicity studies.

b/ At the NOEL, rbc ChE was inhibited by 9% and statistically significant at week 26; the inhibition was 4-11% (not statistically significant) at the end of study.

Overt Toxicity

The lowest NOEL for effects other than ChE inhibition was 0.02 mg/kg/day. This was established both in the 12-month study by Daly (1991) based on peripheral nerve demyelination and in the 25-28 month study by Daly and Hogan (1983) based on abnormal gait, neurotoxicity, and hematological alterations. This is also the same NOEL presented above estimated for brain ChE inhibition. The NOEL of 0.02 mg/kg/day from the study by Daly and Hogan (1983) was also used by USEPA (1998c) in the Reregistration Eligibility Decision Document (RED) to assess the intermediate-term (subchronic) and chronic risk of methyl parathion exposures.

Conclusions

The chronic NOELs for ChE inhibitions were: 0.19 mg/kg/day for plasma ChE inhibition in Sprague-Dawley rats; 0.01 mg/kg/day for RBC ChE inhibition in Wistar rats; and 0.02 mg/kg/day for brain ChE inhibition in mice. Since the NOEL for plasma ChE inhibition was generally at or above one order of magnitude than the other NOELs, even for a shorter term of exposure, it would not be used to characterize the risk of chronic exposures. The NOEL for nerve demyelination, neurological signs and hematological effects was 0.02 mg/kg/day.

18.1.4. Oncogenicity weight of evidence

The available oncogenicity bioassays of methyl parathion did not show clear evidence of oncogenicity in rats and mice. The Scientific Review Panel (SRP), at the June 1999 deliberation under the California Toxic Air Contaminant Act, pointed out the need to emphasize that the rodent bioassays (as presented in Section 9, ONCOGENICITY) indicated limited evidence of oncogenicity. This included the marginal increase ($p<0.05$) in thyroid tumors in rats (Bomhard *et al.*, 1981), the uterus and adrenal tumors in rats (Daly and Hogan, 1983) that exceeded historical ranges although not statistically significant, and the apparent increase in lung tumors in mice, although also not statistically significant.

It has generally been recognized that genetic toxicity studies are not short-term oncogenicity tests. However, the positive genotoxicity under laboratory conditions raised the need to further explore the significance of genotoxicity observations to humans and the potential limitations in the power of detection from rodent oncogenicity studies, specifically concerning the *ad libitum* feeding protocol.

Implication of Genotoxicity Potential

Many OPs, including methyl parathion and methyl paraoxon, have also been shown to have alkylating potential and to bind to cellular macromolecules (WHO, 1986; Gallo and Lawryk, 1991). A literature review on the biological significance of these properties is briefly presented in this section. Bedford and Robinson (1972) studied the alkylating potential of OPs. Using a calorimetric assay with a moderate

nucleophile, 4-(*p*-nitrobenzyl)pyridine (NBP), the alkylating potential of approximately 20 OPs and/or their breakdown products was quantitatively compared to two powerful alkylating agents, dimethyl sulphate and methyl methanesulphonate (MMS). Compared to a relative second-order alkylation rate constant of 2400 for dimethyl sulphate and 100 for MMS, the two OPs with the highest rates were dichlorvos (DDVP) at 34 and methyl paraoxon at 15. Methyl parathion had a lower rate of 8. In speculating on the biological significance of the demonstrated alkylating potential, the authors pointed out that the electrophilic potential of the phosphoryl site, strengthened by the *p*-nitrophenolic leaving group, is expected to be greater than the methyl sites. Quantitatively, the reactivity half-life for the phosphoryl site (reflected by the reaction with hydroxide ion) was approximately 2.4-fold shorter than the methyl site (reflected by the reaction with NBP). The authors also pointed out that the overall significance of the spontaneous (i.e., nonenzymatic) electrophilic reaction should be viewed in the context of the concomitant enzymatic reactivities. The biological significance of the electrophilic potential of an OP appeared to be minimal compared to the much greater rates of its enzymatic reactivities, such as reactions with esterases (e.g., ChE), and the hepatic detoxification pathways (see Figure 1) involving GSH and Cytochrome P450.

Covalent binding of ^{14}C- methyl parathion to cellular macromolecules was reported by Bartoli *et al*. (1991) in rats and mice (see also Table 14). Using U-^{14}C methyl parathion, the authors studied the binding to DNA, RNA, and proteins in 6 rats and 24 mice (received phenobarbitone 2 days prior to treatment) 22 hours after a single intraperitoneal injection of 1.31 Fmol/kg ^{14}C-methyl parathion (based on the study description, the reported dose unit of "mmol/kg" appeared to be a typographic error). The calculated dose was 0.345 mg/kg methyl parathion. The results showed that the binding to RNA and proteins was generally greater than to DNA (the difference was mostly within 10-fold). The respective DNA binding in the liver, kidney, and lung were 0.036, 0.01, and 0.03 pmol/mg in rats and 0.057, 0.11, and 0.08 pmol/mg in mice (the unit was presumed to be pmol methyl parathion bound per mg DNA). *In vitro* studies with microsomal and/or cytosolic fractions from liver, kidney, lung, and brain also showed bindings to calf thymus DNA, with most of them statistically significantly greater than the controls at either $p<0.05$ or <0.001 (Bartoli *et al*., 1991).

The overall implication of the electrophilic and genotoxic potential of methyl parathion and methyl paraoxon remains unknown. It should be noted that among the many OPs for which data on genotoxic and alkylating potential were available, DDVP showed a substantial similarity to methyl parathion except that, unlike methyl parathion, rodent bioassays for DDVP demonstrated sufficient evidence of oncogenicity for classifying DDVP as a Class C, probable, human carcinogen (USEPA, 1989, 1996a). Thus, a comparison of the database for the two chemicals helps to illustrate the difficulties in determining the oncogenic potential based solely on the auxiliary information (e.g., alkylation and genotoxicity potential) when in the absence of evidence in rodent bioassays. Firstly, the study by Bedford and Robinson (1972) presented above showed that the second-order rate constant of alkylation was approximately 2.3-fold higher for DDVP than for methyl paraoxon. Secondly, a study on DNA-binding of DDVP similar to the study by Bartoli *et al*. (1991) for methyl parathion was also available for comparison. Segerback (1981) studied the binding of DDVP to DNA in the soft tissues

of male mice 5 hours after receiving intraperitoneal injection of 1.9 Fmol/kg methyl-^{14}C-DDVP (the calculated dose of 0.420 mg/kg). The alkylation of guanine-N-7 in DNA measured by the radiolabel was 8 x 10^{-13} mol DDVP/g DNA, or 0.0008 pmol/mg DNA. This level is lower than the level reported by Bartoli *et al.* (1991) for methyl parathion. The shorter time after the chemical exposure might have contributed to the lower binding. Finally, both methyl parathion and DDVP showed sufficient evidence of genotoxic potential. On the other hand, only DDVP showed sufficient evidence in animal studies to support the carcinogen classification and for generating the cancer potency (the slope of the dose-response relationship at the low dose range) for a quantitative characterization of risk. The lack of clear evidence of oncogenicity under the experimental conditions utilizing sufficiently high dose levels precluded any further characterization of the oncogenicity potential of methyl parathion.

Effects of *Ad Libitum* feeding

Trends of decreased longevity and increased body weight, degenerative changes, and spontaneous tumors over the last two decades have been widely noted in rodents commonly used in oncogenicity studies (Hart, 1995; Allaben *et al*., 1996; Keenan *et al*., 1997). Among the potential contributing factors to these changes is their consistent association with the high caloric intake under the *ad libitum* (AL) feeding protocol (Keenan *et al.*, 1996; Masoro, 1998). The effects of AL feeding on tumor onset and incidences have been studied through restricting food intake (up to 50% reduction of the AL intake rates) and through varying dietary compositions (e.g., fat, fiber, proteins, total calories). The most commonly noted tumor sites affected by the AL feeding are: hepatocellular tumors in male mice; lymphoma, pituitary and thyroid neoplasms in female mice; and pituitary tumors, adrenal pheochromocytoma, pancreatic islet cell tumors, leukemia, testicular interstitial cell tumors, and mammary gland tumors in rats (Blackwell *et al*., 1995; Higami *et al*., 1994, 1995; Keenan *et al*., 1996; Rao *et al*., 1996; Thurman *et al*., 1994). AL feeding has also been reported to increase chronic degenerative conditions such as nephropathy in rats (Keenan *et al*., 1997).

The effects of AL feeding on the survival and spontaneous tumor development raised a crucial concern that the AL protocol could result in "false-negative" outcomes in rodent oncogenicity tests. The power to detect treatment-related oncogenic effects may be compromised due to the earlier onset of tumors, higher background incidences, lower survival and shorter time for observation due to early death, and the possibility of lower tumor rates at the higher dose groups when the body weight was lower due to lower food consumptions. These concerns were the subjects of many international discussions which consistently pointed to the need for a consensual guidance for dietary control in long term rodent toxicity studies (Allaben *et al*., 1996). Two plausible approaches to diet control include uniform diet restriction (e.g., 70-75% AL consumption) and controlling diets to a pre-determined body weight for all dose groups (Haseman *et al*., 1997; Keenan *et al*., 1998).

The current data on methyl parathion are insufficient for characterizing the impact of AL feeding on the oncogenicity tests. While there were significant changes in food consumption in the high dose groups, they were neither consistently lower than the controls nor positively correlated to the body weight. In

the two rat studies by Bomhard *et al*. (1981) in Wistar rats and Daly and Hogan (1983) in Sprague-Dawley rats, the food consumption at the high dose (50 ppm methyl parathion in the diet) was approximately 15% higher than the controls while the body weight was lower by 8-9%. Conversely, the study in B6C3 F1 mice (Eiben, 1991) showed a 13-15% lower food consumption at the high dose (50 ppm in the diet) while the body weight was higher than the control by 10-20%. In these cases, treatment-related toxicities would also have significant contribution to the changes in these physiological parameters. Ironically, a 10% lower body weight is one of the criteria that define the maximum tolerated dose (MTD) generally required to be included as the highest dose level in oncogenicity studies (McConnell, 1989). Without further studies, the complexity in the relationship between food consumption and body weight in the context of chemical toxicity precludes further considerations of any possible impact of AL feeding to the oncogenicity of methyl parathion.

18.1.5. Toxicity of methyl paraoxon

In the ambient environment, methyl parathion is oxidatively converted to toxicologically active methyl paraoxon. Methyl paraoxon has been concomitantly detected with methyl parathion in the ambient air. Therefore, it is essential to establish a toxicity equivalence factor (TEF) for addressing the total risk from the exposures to both methyl parathion and methyl paraoxon. A comparison of the toxicity of methyl parathion and methyl paraoxon is available in the study by Miyamoto *et al*. (1963b). The toxicity comparison can be based on two criteria; the median lethal dose (LD_{50}) and the dose for 50% inhibition of plasma and brain ChE one hour after i.v. dosing. The term ID_{50} (50% ChE Inhibition Dose) is sometimes used for the latter. The data on LD_{50} were presented in Table 1 for methyl parathion and Table 2 for methyl paraoxon. The data on ID_{50} were presented in Table 3. These data are summarized in Table 26. Based on data in rats and mice, methyl paraoxon could be as much as 8-fold more acutely toxic than methyl parathion. On the other hand, data in guinea pigs indicated a potential for a much greater relative toxicity of methyl paraoxon, as much as 23-fold greater than methyl parathion.

In establishing the TEF, it is important to consider the species sensitivity and the database to which the TEF would be applied. As presented in Section 5.1., *Interspecies and Inter-individuals Sensitivity*, and also indicated in Table 26, rats are generally more sensitive than guinea pigs. It is also important to note that the rat was the laboratory animal species from which the NOELs were selected for use in characterizing the risk of methyl parathion exposures. Based on the above consideration, it was concluded that data from rats instead of guinea pigs were more pertinent for a TEF determination. Based on the range of the methyl parathion-to-methyl paraoxon ratio of 4.5-8.2, a rounded up TEF of 10 for methyl paraoxon was used in this assessment.

In this document, when estimating the overall risk from concomitant exposures to both methyl parathion and methyl paraoxon, it is assumed that the toxicities from the two chemicals are additive. Therefore, the exposure to methyl paraoxon is first converted to a methyl parathion equivalent (MP-equivalent) by

multiplying with the TEF of 10. This MP-equivalent is then added to the exposure of methyl parathion for an estimated "total exposure". Finally, when characterizing the risk, the total exposure is then compared to the toxicity criteria (i.e., NOELs) determined for methyl parathion. Although the TEF was determined based solely on the acute toxicity data, without data to indicate otherwise, it is assumed that the same TEF is applicable to the toxicities for longer terms of exposure (i.e., subchronic, chronic exposures). This is a reasonable assumption especially for toxicity endpoints that are mediated through the mechanism of ChE inhibition. However, data are unavailable to validate this assumption.

Table 26. A comparison of acute toxicity criteria of methyl parathion (MP) and methyl paraoxon (MPoxon) based on data from Miyamoto *et al.* (1963b).

Species/Sex		Toxicity Criteria (mg/kg) Endpoints	MP	MPoxon	MP/MPoxon ratio
Rat		Oral LD_{50}	24.5	4.5	5.4
Male	i.v. LD_{50}	4.1	0.5	8.2	
		i.v. ID_{50}, plasma ChE	1.8	0.4	4.5
		i.v. ID_{50}, brain ChE	2.1	0.3	7.0
Mouse Male/Female		Oral LD_{50}	17	10.8	1.6
Guinea Pig		Oral LD_{50}	417	83	5.0
Male		i.v. LD_{50}	50	2.2	22.7
		i.v. ID_{50}, plasma ChE	24	2.0	12.0
		i.v. ID_{50}, brain ChE	28	1.5	18.7

Abbreviations: i.v., intravenous; LD_{50}, median lethal dose; ID_{50}, 50% ChE inhibition dose (in this, the values reflected the ChE inhibition one hour after i.v. dosing).

18.2. RISK ANALYSIS - Exposure Assessments

The estimates of ambient air and application site exposures resulting from the use of methyl parathion were presented in *Part B of the Evaluation of Methyl Parathion as a Toxic Air Contaminant*. As described above, the total exposure to both methyl parathion and methyl paraoxon is calculated by using a TEF of 10 to convert the exposure of methyl paraoxon to a MP-equivalent. Ideally, and more crucially for acute exposures, this MP-equivalent would be calculated for each sampling day rather than from the summary data as presented in Part B. Nevertheless, the total exposure estimated for the highest single day sample (Part B, Table 2, May 13 dataset) was within the same range as the total exposure calculated from the 95th percentile air concentration from which the ADD was calculated. It was also pointed out in Part B that with the air monitoring samples in which both chemical species were detected, there was an apparent pattern showing that the concentration of methyl paraoxon was approximately 25% of the concentration of methyl parathion. Therefore, it is reasonable to calculate a total exposure using the summary exposure values (i.e., ADD, SADD, AADD) presented in Part B.

The exposures were calculated for three representative subpopulations with default body weights: 6 years old children of 22.6 kg, adult males of 76.9 kg, and adult females of 62.4 kg. Based on the USEPA Exposure Factor Handbook (USEPA, 1996b), the respective default breathing rates of 16.7, 21.4, and 11.4 m^3/day for the three population subgroups were used in the calculation of the absorbed dose.

18.2.1. Ambient air exposures

As presented in Part B, the Absorbed Daily Dose (ADD) of a single day exposure was calculated based on the 95th percentile of the daily concentrations of methyl parathion and methyl paraoxon in the air. The samples in which no residue was detected were assumed to contain residues at 50% of the detection limits (0.10 and 0.25 ng/m^3 for methyl parathion and methyl paraoxon, respectively). The Seasonal Average Daily Dosage (SADD) at a given location was calculated assuming a daily exposure pattern throughout an entire season of use and at the level reflected by the average daily air concentration. The Annual Average Daily Dosage (AADD) was calculated also based on the average daily air concentrations and assumed nine months of exposures in a year (i.e., 9 months of exposure amortized over a year).

Of the four sites in Colusa and Sutter counties for which ambient air monitoring were conducted by Seiber *et al.* (1987), the Maxwell site in Colusa county had the highest estimated exposure. Data from Maxwell site was used in this assessment to represent a realistic high end of ambient air exposures in California that can potentially occur as a result of methyl parathion use. The total exposures for the three representative population subgroups are summarized in Table 27.

Table 27. The total exposure to methyl parathion and methyl paraoxon in the ambient air for three representative population subgroups.

Population subgroups	Exposures (ng/kg/day)[a] MP	MPoxon	Total Exposure[b]
Absorbed Daily Dosage (ADD)[c]			
Child - 6 years old	21.95	4.26	64.55
Adult - Males	8.27	1.60	24.27
Adult - Females	5.43	1.05	15.93
Seasonal Average Daily Dosage (SADD)[d]			
Child - 6 years old	6.24	1.34	19.64
Adult - Males	2.35	0.51	7.45
Adult - Females	1.54	0.33	4.84
Annual Average Daily Dosage (AADD)[e]			
Child - 6 years old	4.68	1.01	14.78
Adult - Males	1.76	0.38	5.56
Adult - Females	1.16	0.25	3.66

a/ The MP (methyl parathion) and MPoxon (methyl paraoxon) data were taken from Part B, Table 5 for the Maxwell site in Colusa County. They were based on the monitoring data by Seiber *et al.* (1987). Values represented the realistic high end of exposures. The exposures were calculated for a 22.6 kg 6 years old child, a 76.9 kg male adult, and a 62.4 kg female adult based on the respective default breathing rates of 16.7, 21.4, and 11.4 m^3/day.

b/ The exposure of MPoxon was multiplied by the toxicity equivalence factor (TEF) of 10 and added to the exposure of methyl parathion. Thus, the total exposure is in MP-equivalence.

c/ The ADD was calculated based on the 95th percentile of daily concentrations, assuming that the residue was at 50% of the minimum detection limit for samples in which no residue was detected.

d/ The SADD was calculated based on the average daily concentrations over the monitored period.

e/ The AADD was calculated based on 9 months of exposure at the SADD amortized over a year.

18.2.2. Application site exposures

The exposure to methyl parathion and methyl paraoxon at the application sites was estimated at 17 and 20 yards from the edge of an application rice field based on the results of air monitoring studies conducted by the ARB (1989) and Seiber and McChesney (1987). Details of these studies were presented in Part B. Only acute exposures to methyl parathion were estimated since seasonal and chronic exposures were not expected at the application site (Part B). No data on methyl paraoxon were available. The total exposure to both methyl parathion and methyl paraoxon was estimated using the TEF of 10 for methyl paraoxon and assuming that the concentration of methyl paraoxon in the air was approximately 25% of the concentration of methyl parathion. This means that the exposure to methyl paraoxon would be equivalent to 2.5- fold of the exposure to methyl parathion. In other words, the total exposure equals to the exposure of methyl parathion multiplied by 3.5. The data are summarized in Table 28.

Table 28. The total acute exposure to methyl parathion and methyl paraoxon at the application sites for three representative population subgroups.

Population subgroups	Absorbed Daily Dosage (ADD) (Fg/kg/day)					
	17 yards			20 yards		
	MP[a]	MPoxon (in MP-eq)[b]	Total[c]	MP[a]	MPoxon (in MP-eq)[b]	Total[c]
Child - 6 years old	0.360	0.90	1.26	0.159	0.40	0.56
Adult - Males	0.136	0.34	0.48	0.060	0.15	0.21
Adult - Females	0.089	0.22	0.31	0.039	0.098	0.14

a/ The MP (methyl parathion) data were taken from Part B, Table 6.

b/ The exposure of MPoxon (methyl paraoxon) was estimated as the MP-equivalence. It was calculated as the ADD of MP exposure multiplied by 2.5. The factor of 2.5 was due to a toxicity equivalence factor (TEF) of 10 for MPoxon and assuming that the air concentration of MPoxon was 25% of the concentration of MP.

c/ The sum of exposures to MP and MPoxon. It is in MP-equivalence.

18.3. RISK ANALYSIS - Risk Characterization

The risk of exposures to methyl parathion and methyl paraoxon was characterized based on non-oncogenic effects. The margin of exposure (MOE) was calculated as the ratio of the NOEL to the estimated exposure. Using the NOELs established in Section 18.1., and the estimated exposures from Table 27 and 28, the MOEs for all exposure scenarios are presented in Table 29. Because of the higher exposures of children due to their higher breathing rates per body weight, the MOEs for children are the lowest in all exposure scenarios. The uncertainties associated with each component of the MOE calculation is described in Section 18.4, RISK ANALYSIS - Risk Appraisal.

18.3.1. Acute toxicity

Two NOELs were used to calculate the MOEs:

1) A human NOEL of 0.31 mg/kg/day (Rider *et al*., 1970, 1971) based on 23% inhibition of plasma ChE and 55% inhibition of RBC ChE which occurred at the LOEL of 0.34 mg/kg/day after 30 days of oral dosing, and

2) A single-dosing NOEL of 0.025 mg/kg/day in rats (Minnema, 1994a) based on severe ChE inhibitions (plasma, RBC, brain) and peripheral nerve demyelination at the LOEL of 7.5 mg/kg/day and some indication of demyelination at the NOEL.

As presented in Table 29, the MOEs for ambient air exposures were 4,800 - 19,000 based on the human NOEL and 390 - 1,600 based on the NOEL in rats. For the application sites, the MOEs were 250 - 1,000 based on the human NOEL. Based on the estimated NOEL in rats, the respective MOEs were 20 and 40 for locations 17 and 20 yards from the application fields.

18.3.2. Subchronic toxicity (from Seasonal Exposures)

Four subchronic NOELs were used to calculate the MOEs for the 9-month seasonal exposures:

1) A human NOEL of 0.31 mg/kg/day (Rider *et al*., 1970, 1971) based on 24% inhibition of plasma ChE and 55% inhibition of RBC ChE occurred at the LOEL of 0.34 mg/kg/day after 30 days of oral dosing,

2) An estimated NOEL of 0.003 mg/kg/day based on a LOEL of 0.03 mg/kg/day in dogs showing 19% plasma ChE inhibition (Daly, 1989)

3) A NOEL of 0.029 mg/kg/day based on 28% RBC ChE inhibition at the LOEL of 0.29 mg/kg/day (Minnema, 1994b),

Table 29. Margins of exposure (MOEs) for methyl parathion and methyl paraoxon present in the air[a].

Exposure scenarios	NOEL (µg/kg/day)	Children 6 yrs old Exposure (µg/kg/day)	Children 6 yrs old MOE	Adults - Males Exposure (µg/kg/day)	Adults - Males MOE	Adults -Females Exposure (µg/kg/day)	Adults -Females MOE
Ambient Air Exposure							
Acute							
310[b] (H; pl, rbc ChE)		0.065	4,800	0.024	13,000	0.016	19,000
25[c] (R; pl, rbc, br ChE; neuro)	0.065		390	0.024	1,000	0.016	1,600
Seasonal							
310[b] (H, pl, rbc ChE)		0.020	16,000	0.0075	41,000	0.0048	65,000
3[d] (D; pl ChE)		0.020	150	0.0075	400	0.0048	630
29[e] (R; rbc ChE)		0.020	1,500	0.0075	3,900	0.0048	6,000
20[f] (R; br ChE; neuro)		0.020	1,000	0.0075	2,700	0.0048	4,200
Chronic							
10[g] (R; rbc ChE)		0.015	670	0.0056	1,800	0.0037	2,700
20[h] (M; br ChE; R; neuro, hem)		0.015	1,300	0.0056	3,600	0.0037	5,400
Application Site Exposure							
Acute; 17 yards							
310[b] (H; pl, rbc ChE)		1.26	250	0.48	650	0.31	1,000
25[c] (R; pl, rbc, br ChE; neuro)	1.26		20	0.48	50	0.31	80
Acute; 20 yards							
310[b] (H; pl, rbc ChE)		0.56	550	0.21	1,500	0.14	2,200
25[c] (R; pl, rbc, br ChE; neuro)	0.56		40	0.21	120	0.14	180

a/ The MOE was calculated as the ratio of the NOEL to the exposure. The endpoints were given in the parenthesis after the NOELs: ChE, cholinesterase inhibition; pl, plasma; rbc, red blood cell; br, brain; H, humans; D, dogs; R, rats; M, mice; neuro, neurotoxicity; hem, hematological effects.

b/ Based on 24% inhibition of plasma ChE and 55% inhibition of RBC ChE occurred at the LOEL of 340 µg/kg/day (0.34 mg/kg/day) after 30 day oral exposures in humans.

c/ Based on severe ChE inhibitions and nerve demyelination at the LOEL of 7.5 mg/kg/day and some indication of demyelination at the NOEL.

d/ Estimated from the LOEL of 0.03 mg/kg/day in dogs showing 19% plasma ChE inhibition.

e/ Based on 28% RBC ChE inhibition at the LOEL of 0.29 mg/kg/day in rats.

f/ Estimated both from the LOEL of 0.2 mg/kg/day in young rats showing $24% ChE inhibition in 5 regions of brain, as well as the LOEL of 0.22-0.44 mg/kg/day for neurobehavioral effects in rats.

g/ Estimated from the LOEL of 0.09 mg/kg/day in rats showing 17% RBC ChE inhibition.

h/ Represented the NOEL both estimated from the LOEL of 0.2 mg/kg/day in mice showing 19% brain ChE inhibition and the NOEL established in rats based on nerve demyelination, abnormal gait, and hematological alterations in two rat studies.

4) An estimated NOEL of 0.02 mg/kg/day based on a LOEL of 0.2 mg/kg/day in young rats showing $24% ChE inhibition in 5 regions of brain (Kumar and Desiraju, 1992), as well as a LOEL of 0.22-0.44 mg/kg/day showing neurobehavioral effects (Schulz *et al*., 1990).

As presented in Table 29, the lowest MOEs for ambient seasonal exposures were 16,000 based on the human NOEL, 150 based on plasma ChE inhibition, 1,500 based on RBC ChE inhibition, and 1,000 based on brain ChE inhibition and neurobehavioral effects.

18.3.3. Chronic toxicity

Two NOELs were used to calculate the MOEs:

1) An estimated NOEL of 0.01 mg/kg/day based on a LOEL of 0.09 mg/kg/day in rats showing 17% RBC ChE inhibition (Bomhard *et al*., 1981), and

2) A NOEL of 0.02 mg/kg/day, estimated from a LOEL of 0.2 mg/kg/day in mice showing 19% brain ChE inhibition (Eiben, 1991). The same NOEL was also established in rats based on abnormal gait and hematological alterations, and nerve demyelination in two rat studies (Daly and Hogan, 1983; Daly, 1991) .

As presented in Table 29, the lowest MOEs for ambient seasonal exposures were 670 based on RBC ChE inhibition, and 1,300 based on brain ChE inhibition and neurological and hematological effects.

18.4. RISK ANALYSIS - Risk Appraisal

Inherent in each risk assessment are uncertainties associated with the availability and use of data in assessing the potential risk of health hazards. Assumptions are made when the existing data are limited or insufficient for identifying the potential hazards and characterizing the dose-response relationship and the exposures. Uncertainties also exist when data from experimental animals are extrapolated to humans in characterizing the risk. Major assumptions and the associated uncertainties in this assessment of ambient air exposures to methyl parathion and methyl paraoxon are highlighted in this section.

18.4.1. Toxicity assessment

The NOELs determined from oral studies were used to assess the potential risk of inhalation exposures in the air. Potential uncertainties are introduced in the route-to-route extrapolation. For the acute toxicities, the existing toxicity data in experimental animals indicated that the extent of absorption is comparable between the oral and inhalation routes and that the toxicity manifestations are similar between the two routes based on the total exposure over a few hours. Comparative data are

unavailable regarding the dose-response relationship between daily bolus oral dosing (e.g., gavage or capsule dosing) and inhalation exposures beyond a few hours (e.g., 24-hour daily exposure). Therefore, the default assumption of equivalent toxicity based on the total daily dose is applied to all route-to-route extrapolation scenarios in this assessment. Without data in humans, the cross-route equivalence of dose is generally also assumed for humans.

Additional uncertainty was introduced when toxicity data are extrapolated from animals to humans. Without sufficient evidence to indicate otherwise, the current default assumption is that humans can be 10-fold more sensitive to methyl parathion and paraoxon than the most sensitive animal tested. Using this assumption to extrapolate toxicological data from animals to humans would introduce a greater uncertainty than when using a NOEL directly established in humans. Although a 30-day NOEL can be established from a series of studies in humans, the limited nature of these studies could not support the exclusive use of the human NOEL for assessing the risk of acute and subchronic exposures.

In addition to these common uncertainties often necessary in risk assessment (e.g., route-to-route and interspecies extrapolations), there were uncertainties associated with the specific database from which the NOELs were determined. These are presented in the following discussions.

Acute Toxicities

Two NOELs were used to calculate the MOEs for the acute exposures, each with its own set of uncertainties. The NOEL of 0.31 mg/kg/day was based on studies in humans. This 30-day NOEL in humans was established from a series of studies by Rider *et al*. (1970, 1971). Its use in characterizing the risk of acute exposures may err on the conservative side. On the other hand, there are considerable uncertainties with respect to the adequacy of this NOEL for risk assessment. The scope of the study was limited to the inhibition of plasma and RBC ChE activities in five adult test subjects per dose group and two control test subjects. The results were reported only as abstracts (<200 words) for platform presentations in scientific meetings with no subsequent journal publications. While no effects were found at the 0.31 mg/kg/day, two of the five subjects at the LOEL of 0.34 mg/kg/day had significant depression of ChE activities. The highest inhibition was 23% for plasma ChE and 55% for RBC ChE. It should also be noted that this LOEL was only 10% higher than the NOEL and that the dose can only be estimated using a default assumption of a 70 kg (154 pounds) body weight. A higher body weight for the test subjects would correspond to a lower calculated NOEL.

The other NOEL used for assessing the risk of acute exposures was determined in rats. The use of an animal NOEL necessitates an interspecies extrapolation. However, unlike the human studies, many endpoints that were potentially more sensitive were investigated (e.g., brain ChE, neurohistopathological and neurobehavioral observations). Specific to the NOEL of 0.025 mg/kg/day used in this assessment, an argument might be made that a fine-tuning to raise the NOEL is possible in light of the 300-fold difference in between the LOEL and the NOEL. However, the possibility for a higher NOEL should be viewed in light of the effects noted at the NOEL. These included a 22%

plasma ChE inhibition, although not statistically significant, and an apparent increase in demyelination of dorsal and ventral lumbar root fibers. The degree of any possible conservativeness associated with the NOEL should also be viewed in the context of the sizable toxicological database which showed the estimated or established NOELs generally clustered within the range of 0.003-0.02 mg/kg/day for all endpoints and durations.

Subchronic Toxicities

Again, both NOELs determined in humans and laboratory animals were used in characterizing the risk of seasonal exposures (up to 9-month) since the human NOEL was not sufficient to address all endpoints reported in the database. Unfortunately, the NOELs for the plasma and brain ChE inhibition and the neurobehavior effects from the laboratory animals were estimated from LOELs which represented the lowest dose tested in each study that showed the most sensitive endpoints and/or species. Uncertainties were introduced when a default factor of 10 was used to estimate a NOEL from the LOEL. As discussed earlier, this default does not account for the shape of the dose response relationship and the severity of effects at the lowest dose tested. Nevertheless, the estimated NOEL of 0.02 mg/kg/day for brain ChE inhibition and neurobehavioral effects in Wistar rats were supported by the slight increase in the incidence of alopecia and skin sores noted at 0.029 mg/kg/day in Sprague-Dawley rats reported by Minnema (1994b).

It should also be noted that, except for the lower NOEL estimated for the plasma ChE inhibition, the subchronic NOELs were generally in the same range as the chronic NOEL. Regardless of the toxicological considerations and uncertainties underlying the estimated subchronic NOEL, having the same NOEL for both subchronic and chronic exposures appears to be prudent, especially when the potential season of exposure is 9 months of a year. Alternatively, there are no data to assure that the 30-day NOEL determined in humans, with its underlying limitations and uncertainties, would be adequate for evaluating the risk of a much longer exposure period.

Chronic Toxicities

There was relatively firm support for the chronic NOEL of 0.02 mg/kg/day for neurotoxicities and hematological effects. It was the same level determined from the two available studies in Sprague-Dawley rats. It was also the same level that could be estimated from the LOELs for other effects. These included the LOELs of 0.2 mg/kg/day for brain ChE inhibition in mice (Eiben, 1991) and the LOEL of 0.22 mg/kg/day for effects in the cortex of Wistar rats reported in the 3-generation study by Nagymajtenyi *et al*. (1995). Comparatively, the uncertainty was greater for the NOEL of 0.01 mg/kg/day for the RBC ChE inhibition since it was estimated based on a LOEL.

Toxicity of methyl paraoxon

Uncertainties exist regarding the toxicity of the degradation product methyl paraoxon. The pesticidal use of methyl parathion results in the presence of not only methyl parathion but also methyl paraoxon in the air. A TEF of 10 for methyl paraoxon was used to assess the total risk of exposures to both chemicals. The exposure to methyl paraoxon was multiplied by the TEF and then added to the exposure of methyl parathion. Uncertainties were introduced in estimating the toxicity of methyl paraoxon relative to methyl parathion. The limited acute toxicity data showed that the TEF could be up to 8-fold higher in rats but as much as 23-fold higher in guinea pigs. However, the same acute toxicity data also showed that rats were generally more sensitive than guinea pigs. The final TEF of 10 was rounded up from the TEF of 8 in rats. Additional uncertainties were also introduced when the same TEF was applied to all exposure durations. However, data for comparing genotoxicity and the toxicities of intermediate and long terms of exposures were unavailable to validate this assumption.

18.4.2. Exposure assessment

The ambient air exposure levels used in this assessment were estimated based on the monitoring study conducted for rice applications during May-June, 1986, in Sutter and Colusa counties. Of the four monitoring sites, the air concentration was the highest at Maxwell, Colusa County. The exposures at this site were used in this assessment to represent the realistic high end of the potential exposures.

Uncertainties in the exposure estimates are typically introduced when default body weights and breathing rates rather than the individually correlated parameters are used. Uncertainties are also introduced when the monitoring data over a period of one month are extrapolated over a year based on 9 months per year of exposure frequencies. Furthermore, the inter-individual variation and the variabilities of concentrations in the air are lost when the exposure is expressed as a point estimate using default exposure parameters instead of a population distribution. Aside from these common sources of uncertainties, three specific areas of uncertainties are highlighted in this section.

First, there are the uncertainties due to the limitations in the available data to account for the dynamic changes in air concentrations. Several factors are expected to contribute to the variable methyl parathion and methyl paraoxon levels in the air. These include the dynamics of meteorological factors, the geographic locations and temporal patterns of use (especially pertaining to the multiple contributing sources in an area wide setting), and the application rates and methods for each specific usage (especially pertaining to the movement to off-site locations). Although the monitoring studies were designed to capture a temporal and spatial window that represented a potential high end of exposure, not all of these factors can be accounted for within the existing data.

Secondly, the exposure assessment for both the ambient air and application site exposure was estimated based on the air monitoring data associated with rice applications. While the total use of methyl parathion on rice generally represented the highest among all crops in California (Part A,

Environmental Fate, Table 6) , the total amount of methyl parathion applied during the months of ambient air monitoring (i.e., May-June) was not consistently higher in Colusa county from which the ambient air exposure was modeled (Part A, *Environmental Fate*, Table 5). Nor was the application rate to rice fields the highest rate for all crops. It should also be noted that the pattern of dissipation and dispersion from the applications to rice fields could be substantially different from applications to other crops. Therefore, the exposures estimated from these limited monitoring studies may not capture the highest potential exposure level associated with agricultural use in California.

Finally, in calculating the total exposure that assumed a TEF of 10 for methyl paraoxon, it was assumed that the concentration of methyl paraoxon was approximately 25% of methyl parathion. Additional uncertainties are introduced due to the dynamic fluctuation in the ratio of methyl parathion to methyl paraoxon in time and space. The apparent greater toxicity of methyl paraoxon would mean that, at a given molecular concentration, an environment with a higher ratio of methyl paraoxon to methyl parathion would be expected to have greater risks. Current data were not sufficient to assure that assuming a concentration of methyl paraoxon at 25% of methyl parathion would be adequate for describing all exposure scenarios of concern.

18.4.3. Risk characterization

In characterizing the potential risk of exposures to methyl parathion in the air, a common default assumption is that, the sensitivity among human population could vary as much as 10-fold. Many factors can potentially contribute to individual variations. Among these are genetic predisposition, age, gender, environmental factors, and health and nutritional statuses. Some data on the variations of several biochemical markers are available. However, there is insufficient information for quantitatively extrapolating these fairly wide variations to the expressed sensitivity among humans. The polymorphic variation of paraoxonase, the paraoxon detoxification enzyme, could be as much as 60-fold within humans (see: Section 5.1., *Interspecies and Inter-individual sensitivity)*. The intra- and inter-individual variations of plasma and RBC ChE have also been documented. Brock and Brock (1990) studied the variation of plasma ChE in 193 healthy volunteers (19-65 years old). The intra-individual variation reportedly ranged from 3 to 42% during the 8-month sampling period. On the other hand, the inter-individual variation appeared to be reflective of the five identified genotypes, including one that showed no detectable plasma ChE (Brock and Brock, 1990). Mutch *et al.* (1992) reported that the activities of RBC AChE, lymphocyte neuropathy target esterase, serum ChE, paraoxonase, and arylesterase varied by 2- to 6.5-fold among 127 Caucasian males. The authors cautioned, however, that the implication of the differences in these marker enzymes on the susceptibility to neurotoxicity is by no means certain since toxicities may not correspond directly to the baseline values of these enzymes (Mutch *et al.*, 1992).

In addition to the data on biomarkers, acute toxicity studies in laboratory animals revealed age-related sensitivity to methyl parathion (see: Section 6.2.3., *Age-related sensitivity*). Based on the LD_{50} (Benke and Murphy, 1975; see Table 1) and ED_{50} for plasma and brain ChE inhibition (Pope and

Chakraborti, 1992; see Table 3), neonates may be 10 times more susceptible to acute methyl parathion toxicity than older rats. No comparative information on age-related susceptibility in humans is available. However, considering the heterogeneity of human population with respect to the various contributing factors in addition to age, the inter-individual sensitivity in a human population may be greater than the default factor of 10 commonly used in risk assessment. This means that the MOE of 10 generally considered as sufficient for the protection of human health when it is calculated based on a NOEL determined in humans may not be sufficient for sensitive population subgroups such as infants and children. This consideration is especially pertinent when the human NOEL was determined based on a study from a small number of adults and that an increase in 10% of dose (from the NOEL of 0.31 to the LOEL of 0.34 mg/kg/day) resulted in marked effects; i.e., from no significant effects at the NOEL to as high as 55% depression of RBC ChE in two of the five test subjects at the LOEL.

In recent years, issues have been raised concerning the scope of pesticide risk assessment to adequately address the risk of pesticide exposures in the environment. The key issues outlined in the 1993 report *Pesticides in the Diets of Infants and Children* by the National Academy of Science (NAS, 1993) included: 1) a more thorough evaluation of the risks of infants and children, 2) the inclusion of all exposures from multiple routes of contact (the "aggregate exposure"), and 3) the risk of concomitant exposures to multiple chemicals (the "cumulative risk"). The federal Food Quality Protection Act of 1996 (FQPA) subsequently incorporated these areas as provisions under the FFDCA (Federal Food, Drug and Cosmetic Act). Although these concerns were raised regarding pesticide exposures, particularly in the food, they have far reaching implications beyond pesticides and impacting the general practices and policies in risk assessments. The implementation of these provisions is still undergoing debate and refinement. The following discussions on these issues were specifically focused on the risk assessment of methyl parathion.

With respect to the sensitivity of infants and children, it is uncertain whether the factor of 10, traditionally assumed for the inter-individual difference in sensitivity, is adequate when the acute and seasonal MOEs were calculated from a 30-day NOEL of 0.31 mg/kg/day determined from the study by Rider *et al*. (1970). The FQPA requires the considerations of an additional safety factor of up to 10 to account for pre- and post-natal toxicity and the completeness of the database. The toxicological database in rats showed that neonates can be up to 10-fold more sensitive than the adults (see: Section 6.2.3.). The extent of pre- and post-natal sensitivity can also be further evaluated based on the completed submission of toxicity studies required under the Senate Bill 950 (SB950), particularly the studies on developmental and reproductive toxicities. A possible higher sensitivity was indicated in the developmental toxicity studies. The studies in rats by Gupta *et al*. (1985) and Becker *et al*. (1987) showed that fetal toxicities (ossification, survival, body weight, brain ChE activities) occurred at the same dose level of the maternal toxicity (maternal weight gain, survival, brain ChE activities). On the other hand, the rabbit teratology study by Hoberman (1991) reported no clinical signs in the dams while fetal effects of thickened areas of rib ossification were noted at the LOEL of 9 mg/kg/day, although there were substantial RBC ChE inhibitions in the dam at this LOEL. In addition to the developmental toxicity studies, the reproductive toxicity data also suggested a higher sensitivity through pre-natal

exposures. In the two available studies, a reduction of pup survival consistently occurred at the LOELs on day 4 (Daly and Hogan, 1982) and up to week 4 (Loser and Eiben, 1982) after birth. In the study by Daly and Hogan (1982), reduction of maternal body weight gain and pup survival occurred at the same LOEL of 2.3 mg/kg/day. However, in the study by Loser and Eiben (1982), a reduction of pup survival was noted at the LOEL of 0.71 mg/kg/day in the absence of any report of maternal toxicities. In conclusion, the entire database showed a possibility of higher sensitivity from pre- and post-natal exposures. In light of the differential sensitivity, and the observed neurotoxicity, USEPA determined that a study on developmental neurotoxicity was necessary (USEPA, 1998c). Meanwhile, an additional safety factor of 10 should be used in calculating both the acute and the chronic Reference Dose (RfD) (USEPA, 1998c).

This assessment evaluates the risk of methyl parathion and methyl paraoxon in the air through the inhalation pathway. The agricultural use of methyl parathion is expected to result in significant exposures from additional pathways such as residues in food, and dermal exposures through occupational contacts. The risk of aggregate exposure is routinely addressed in the DPR risk characterization document (RCD) conducted under the mandate of Senate Bill 950 (SB950, Birth Defect Prevention Act). The RCD for methyl parathion that includes dietary and occupational exposures will be conducted subsequent to this evaluation mandated under the AB1807.

The general pattern of pesticide use also indicated the need to address the risk of concomitant exposure to other organophosphate chemicals through the same mechanism of toxicity. However, an approach to realistically address the cumulative risk of chemicals with a common mechanism of toxicity is presently not available. Related issues have been the subject of a series of discussions under the implementation of the FQPA. The USEPA Office of Pesticide Programs (OPP) policy proposed in a FIFRA Scientific Advisory Panel (SAP) meeting in March, 1998, was that cholinergic toxicities of OPs were expressed through the common mechanism of interactions with ChE, and consequently, the toxicities of OP pesticides should be considered as a group for cumulative risk assessments (OPP, 1998). Nevertheless, many issues critical to a realistic assessment of cumulative risk remain unresolved. Some key issues included the consideration for the interaction among chemicals pharmacokinetically and pharmacodynamically, and the co-existence and dynamic fluctuation of levels of all chemicals in a given time of exposure.

While a scientifically defensible approach to quantitatively estimate the cumulative effects of OPs is not yet available, the general extent of their concomitant use with methyl parathion in California may be indicated from the pattern of their use in California. DPR Pesticide Use Report data showed that multiple OPs have commonly been used in a general location (e.g., county) within a given time period (e.g., month, season, year). This can be illustrated by the pattern of use of OP pesticides in Colusa county, the air monitoring data of which were used in this document to model the human exposures. During 1995 - 1997, the amount of agricultural use of methyl parathion in Colusa county was approximately 2,800-4,000 pounds. It was mainly applied to rice, with only a very small amount used in dried beans (159 pounds in 1996) and sunflower (3 pounds in 1995). On a yearly basis, eight OP

pesticides were used in agricultural applications at or above the range of methyl parathion poundage during the 3-year period in Colusa. They were: acephate, azinphos methyl, chlorpyrifos, dimethoate, malathion, methidathion, naled, and phosmet. Although the pattern of use varied from year to year, all except naled also had substantial use (above 2,000 pounds) over the season of methyl parathion use (April - July) in Colusa county. These OPs were applied widely to various crops, including grapes, vegetable crops (e.g., beans, cabbage, corn, cucumber, kale, kohlrabi, melons, onion, peppers, squash, tomatoes, turnip), tree crops (almond, pear, prune, walnut), and field crops (e.g., alfalfa, cotton, sorghum, sugarbeets, and sunflower). It should also be noted that specific to the use of OPs on rice in Colusa county, data for the most 3 recent years showed that within the 4 typical months of methyl parathion use season, the use of malathion varied within 2-fold of the amount of methyl parathion applied (2,700-3,800 pounds for methyl parathion versus 1,500-6,900 pounds of malathion).

Data are not available for showing the presence of multiple OPs in the air because samples have not been routinely analyzed for more than one pesticide. However, the occurrence of OPs is evident in foods. Nationwide, USDA pesticide data program or PDP has the most extensive data on pesticide residues in agricultural commodities. The program is designed specifically for generating data risk assessment, and samples are analyzed for multiple OPs. The PDP data showed that, of the commodities sampled in California during 1995 and 1996, peach had a high frequency of methyl parathion detection in a total of 177 samples during these two years. Approximately 80 percent of the 47 samples that showed a detected amount of methyl parathion contain two to four OPs.

18.5. RISK ANALYSIS - Reference Concentrations

In general, a MOE of 10 is considered sufficiently protective when using a NOEL determined in humans. A MOE of 100 is considered sufficiently protective when using a NOEL determined in experimental animals. These general benchmark MOEs were used to calculate the reference concentrations of methyl parathion in the air. Reference concentrations are presented in Table 30 for acute, seasonal, and chronic exposures. These concentrations should be viewed in the context of the accompanied uncertainties described under the previous section (Section 18.4., *RISK ANALYSIS - Risk Appraisal*). Particularly, when considering the limitations and uncertainties in the toxicity database and the various issues in risk characterization, it may be prudent to apply higher MOEs to some toxicity criteria than what was used in Table 30. For example, it may be prudent to apply a MOE greater than 10 when calculating the reference concentrations based on the human NOEL.

It is important to note that the database on acute lethality and ChE inhibitions showed an age-related higher sensitivity in young animals.

As mentioned in the previous section, while requiring a study to evaluate the potential for developmental neurotoxicity, USEPA's current policy is to apply the FQPA additional safety factor of 10 in calculating both the acute and chronic RfD (USEPA, 1998c, 1999). This resulted in the use of an overall uncertainty factor of 1,000. The most recent USEPA's RfD for acute, short-, and intermediate- term of

exposure was 0.0001 mg/kg/day (0.1 Fg/kg/day) based on the NOEL of 0.1 mg/kg/day from the 1-year rat study by Daly (1991). This NOEL is 4- to 5-fold higher than the NOELs used in this document for calculating the acute and seasonal MOE and Reference Concentration (RfC). USEPA's chronic RfD was 0.00002 mg/kg/day (0.02 Fg/kg/day) based on the chronic NOEL of 0.02 mg/kg/day determined for ChE inhibition, neurotoxicities, and hematological changes in the study by Daly and Hogan (1983). The same level of NOEL was used in this document (Table 29) for calculating the MOE and RfC for chronic exposures. Applying the additional factor for infants and children will result in RfC's that are 10-fold lower than the values presented in Table 30.

The reference concentrations for a 24-hour period were calculated based on the following equations:

Since,

$$Exposure\ (mg/kg/day) = \frac{Concentration\ (mg/m^3)\ x\ Breathing\ Rate\ (m^3/day)}{Body\ Weight\ (kg)}$$

and that,

$$Reference\ Exposure\ (mg/kg/day) = \frac{NOEL\ (mg/kg/day)}{MOE}$$

Reference concentration is therefore calculated as:

$$Reference\ Concentration\ (mg/m^3) = \frac{Body\ Weight\ (kg)\ x\ NOEL\ (mg/kg/day)}{Breathing\ Rate\ (m^3/day)\ x\ MOE}$$

Table 30. The 24-hour reference concentrations of methyl parathion in the air for acute, seasonal, and chronic exposures[a].

Toxicity Criteria			Benchmark	Reference Concentration of MP			
NOEL	Species	Endpoints	MOE[b]	MP only[c]		MP+MPoxon[d]	
(mg/kg/day)			(Fg/m^3)	(ppt)	(Fg/m^3)	(ppt)	
Acute Exposures							
0.31	Humans	pl, rbc ChE	10	40	4200	12	1200
0.025	Rats	pl, rbc, br ChE, nerve demyelination	100	0.34	30	0.1	10
Seasonal Exposures							
0.31	Humans	pl, rbc ChE	10	40	4200	12	1200
0.003	Dogs	pl ChE	100	0.04	4	0.01	1
0.029	Rats	rbc ChE	100	0.39	40	0.1	11
0.02	Rats	br ChE, neurobehavioral effects	100	0.3	30	0.08	8
Chronic Exposures							
0.01	Rats	rbc ChE	100	0.14	14	0.04	4
0.02	Mice Rats	br ChE peripheral neuropathy, demyelination	100	0.3	30	0.08	8

a/ The reference concentrations are calculated based on the default exposure parameters of a 6 years old child (16.7 m^3/day for a 22.6 kg child) since children have higher breathing rate on a per body weight basis. The conversion factor of air concentration was 1 ppt = 10.1 ng/m^3.

b/ An additional safety factor of 10 should be considered for the demonstrated age-related sensitivity in the acute database and the potential developmental neurotoxicity.

c/ The reference concentration for methyl parathion was calculated with the assumption that only methyl parathion was present in the air.

d/ The reference concentration for methyl parathion was calculated with the assumption that methyl paraoxon is present at 25% of the methyl parathion, and has a TEF of 10. Applying this assumption resulted in a reference concentration of methyl parathion 3.5-fold below the calculated value which did not provide for its degradation to methyl paraoxon.

The reference concentrations presented in Table 30 are calculated based on the default exposure parameters of a 6 years old child (16.7 m^3/day for a 22.6 kg child; see: Section 18.2., *RISK ANALYSIS - Exposure Assessment*) since children have higher breathing rates on a per body weight basis. Two sets of reference concentrations were calculated. One set considers only the presence of methyl parathion while the other set takes into account the presence of methyl paraoxon at 25% of the concentration of methyl parathion. The reference concentration for "methyl parathion only" scenario ranged from 0.34 to 40 Fg/m^3 (or, 0.03 to 4.2 ppb) for a 24-hour exposure, 0.04 to 40 Fg/m^3 (or, 0.004 to 4.2 ppb) for a seasonal exposure, and was 0.14 to 0.3 Fg/m^3 (or, 0.014 to 0.03 ppb) for a chronic daily exposure. Accounting for the concomitant presence of methyl paraoxon reduced the reference concentrations by 3.5-fold, resulting in reference concentrations for methyl parathion as low as 0.1 Fg/m^3 (or, 10 ppt) for the acute exposures and 0.01 to 0.08 Fg/m^3 (or, 1 to 8 ppt) for seasonal and chronic exposures.

It should be noted that the chronic reference concentration is the same level as the level for the seasonal exposures. Regardless of the toxicological considerations and uncertainties underlying the estimated subchronic NOEL, having the same level for both exposure scenarios appears to be prudent, especially when the season for exposure is 9 months of a year and that the alternative reference concentration derived from the human NOEL, with its substantial limitations and uncertainties, was only for a period of 30 days.

18.6. Conclusions

Taking into account the use patterns of methyl parathion in California, the total ambient air exposures (the combined exposure of methyl parathion and methyl paraoxon) were estimated based on the highest ambient air concentrations detected in Colusa and Sutter counties in 1986 during the season of application to rice fields. The MOEs for the acute ambient air exposures were 4,800 - 19,000 based on the NOEL from limited studies in humans, and 390 - 1,600 based on the NOEL established in rats. The MOEs for the ambient seasonal exposures were 16,000 - 65,000 based on the NOEL from limited studies in humans, 1,000 - 4,200 based on the NOEL established in rats, and 150 - 630 based on the NOEL estimated in dogs for plasma ChE inhibition. The MOEs for ambient chronic exposures were 1,300 - 5,400 based on the NOEL established in rats, and 670 - 2,700 based on RBC ChE inhibition in rats. For the exposures at the application site (17 and 20 yards from the rice field), the MOEs were 250 - 1,000 based on the NOEL from limited studies in humans, and 20 - 80 (at 17 yards) based on the NOEL established in rats.

The benchmark MOEs traditionally considered as adequate for the protection of human health are a MOE of 10 based on a human NOEL and a MOE of 100 based on an animal NOEL. Thus, except for the seasonal MOE of 150 (plasma ChE endpoint), the MOEs for the ambient air exposures to methyl parathion are at least 7-fold greater than these benchmarks. The MOEs for 17 and 20 yards from the rice field are below the benchmark of 100 for the protection of human health.

The MOEs presented in this document pertained only to the inhalation exposures from the air and did not include the concomitant exposures through dietary intakes. It is essential that the MOEs should be viewed in the context of the limitations and uncertainties discussed in the previous sections. Additional considerations should also be given to the demonstrated age-related sensitivity and the potential developmental neurotoxicity.

Reference concentrations of methyl parathion in the air were calculated based on the traditional benchmark MOEs for the protection of human health. Accounting for the concomitant presence of methyl paraoxon, the reference concentration of methyl parathion could be as low as 0.1 Fg/m^3 (10 ppt) for the acute exposures and 0.01 to 0.08 Fg/m^3 (1 to 8 ppt) for seasonal and chronic exposures. Again, additional considerations should also be given to the demonstrated age-related sensitivity and the potential developmental neurotoxicity.

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